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CONTENTS.

1. **Diphtheria Carriers.** By J. A. ARKWRIGHT. (*British Medical Journal*, II., 1910.)

2. **Hypersensibility to Pure Egg Albumen.** By H. W. ARMIT. (*Zeitschrift für Immunitätsforschung*, Orig. Bd. 6, 1910.)

3. **The Ætiology and Epidemiology of Paratyphoid Fever and "Food-Poisoning."** By F. A. BAINBRIDGE. (*Proc. Royal Society of Medicine*, February, 1911.)

4. **An Outbreak of Acute Gastro-Enteritis caused by** *B. Paratyphosus (B.)* By F. A. BAINBRIDGE and R. DUDFIELD. (*Journal of Hygiene*, Vol. XI., 1911.)

5. **On the Paratyphoid Group of Bacilli.** F. A. BAINBRIDGE and R. A. O'BRIEN. (*Journal of Hygiene*, Vol. XI., 1911.)

6. **Brief Notes on the Structure and Development of** *Spirochaeta anodontæ Keysselitz.* By W. C. BOSANQUET. (*Quarterly Journal of Microscopical Science*, Vol. LVI., 1911.)

7. **Observations on Leucoprotease and "Anti-Leucoprotease."** By C. H. BURTON BRADLEY. (*Journal of Hygiene*, Vol. X., 1910.)

8. **The Problem of Pulmonary Tuberculosis considered from the Standpoint of Disposition.** By W. BULLOCH and M. GREENWOOD, Junr. (*Proc. Royal Society of Medicine*, 1911.)

9. **The Process of Disinfection by Chemical Agencies and Hot Water.** By HARRIETTE CHICK. (*Journal of Hygiene*, Vol. X. 1910.)

10. **On the "Heat Coagulation" of Proteins.** By HARRIETTE CHICK and C. J. MARTIN. (*Journal of Physiology*, Vol. XL., 1910.)

11. **The Fleas common on Rats in different parts of the World, and the readiness with which they bite man.** By HARRIETTE CHICK and C. J. MARTIN. (*Journal of Hygiene*. Vol. XI., 1911)

12. **An Examination of the Blood Serum of Idiots by the Wassermann Reaction.** By H. R. DEAN. (*Proc. Royal Society of Medicine*, July, 1910.)

13. **Comparison of the Original Wassermann Method with some of its Modifications.** By H. R. Dean. (*British Medical Journal*, II., 1910.)

14. **Recognition of** *B. typhosus* **by Complement Fixation.** By H. R. Dean. (*British Medical Journal*, II., 1910.)

15. **Studies in Complement Fixation with Strains of Typhoid, Paratyphoid, and Allied Organisms.** By H. R. Dean. (*Proc. Royal Society of Medicine*, 1911.)

16. **Synthesis of Dl.-3 : 4-Dihydroxyphenylalanine.** By Casimir Funk. (*Transactions of the Chemical Society*, 1911.)

17. **On the spread of Epidemic Plague through Districts with Scattered Villages.** Part II., **Statistical Analyses of Data respecting Epidemics of Plague in Three Districts of the Punjab.** By M. Greenwood, Junr. (*Journal of Hygiene*, Vol. X., 1910.)

18. **The Fatality of Fractures of the Lower Extremity and of Lobar Pneumonia. A Study of Hospital Mortality Rates, 1751-1901.** By M Greenwood, Junr., and R. H. Candy. (*Journal of the Royal Statistical Society*, Vol. LXXIV., 1911.)

19. **A Biometric Study of Phagocytosis with Special Reference to the "Opsonic Index."** By M. Greenwood, Junr., and J. D. C. White. (*Biometrika*, Vol. VII., 1910.)

20. **Recent Researches on Alcoholic Fermentation.** By A. Harden. (*Journal of the Institute of Brewing*, Vol. XVI., 1910.)

21. **On the Alleged Presence of an Alcoholic Enzyme in Animal Tissues and Organs.** By A. Harden and H. Maclean. (*Journal of Physiology*, Vol. XLII., 1911.)

22. **The Diacetyl Reaction for Proteins.** By A. Harden and Dorothy Norris. (*Journal of Physiology*, Vol. XLII., 1911.)

23. **The Fermentation of Galactose by Yeast and Yeast-juice.** (Preliminary Communication). By A. Harden and R. V. Norris. (*Proc. Royal Society*, B. Vol. 82, 1910.)

24. **The Influence of Salts on the Autofermentation of Yeast.** Preliminary note. By A. Harden and S. G. Paine. (*Journal of the Chemical Society*, 1911.)

25. **Apparatus for collecting and Measuring the Gases Evolved during Fermentation.** By A. Harden, J. Thompson and W. J. Young. (*Bio-Chemical Journal*, 1910.)

26. **Alcoholic Fermentation.** By A. Harden and W. J. Young. (*The Proceedings of the Seventh International Conference of Applied Chemistry, London*, 1909.)

27. **The Alcoholic Ferment of Yeast Juice.** Part VI., **The Influence of Arsenates and Arsenites on the Fermentation of the Sugars by Yeast-juice.** By A. Harden and W. J. Young. (*Proc. Royal Society*, B. Vol. 83, 1911.)

28. **Über die Zusammensetzung des durch Hefepresssaft gebildeten Hexosephosphorsäure.** I. By A. Harden and W. J. Young. (*Biochemische Zeitschrift*, Bd. 32, 1911.)

29. **On the Structure of Complement in Relation to Deviation.** By J. Henderson-Smith. (*British Medical Journal*, II., 1910.)

30. **Report to the Local Government Board on the Enteric Fever "Carrier"; being a Review of Current Knowledge on this Subject.** By J. C. G. Ledingham. (*Appendix to the Report of the Medical Officer of the Local Government Board*, 1910.)

31. **Dysentery Carriers.** By G. H. K. Macalister. (*British Medical Journal*, II., 1911.)

32. **The Physiological Mechanism of Anaphylactic Shock.** A Preliminary communication. By W. H. Manwaring. (*Johns Hopkins' Hospital Bulletin*, Vol. XXI., 1910.)

33. **Serophysiologische Untersuchungen. I. Der Physiologische Mechanismus des Anaphylaktischen Shock.** By W. H. Manwaring. (*Zeitschr. f. Immunitätsforchung*, Orig. Bd. 8, 1911.)

34. **Serophysiologische Studien. II. Mitteilungen über die Beziehungen zwischen dem anaphylaktischen Shock und dem Peptonshock bei Hunden.** By W. H. Manwaring. (*Zeitschr. f. Immunitätsforchung*, Orig. Bd. 8, 1911.)

35. **Observations on Rat Plague in East Suffolk,** November and December, 1910. By C. J. Martin and S. Rowland. (*Appendix to the Report of the Medical Officer of the Local Government Board*, 1911.)

36. **Report on a Collection of Blood-Parasites made by the Sleeping Sickness Commission, 1908-9, in Uganda.** By E. A. Minchin. (*Reports of the Sleeping Sickness Commission of the Royal Society*, 1910.)

37. **Some Problems of Evolution in the Simplest Forms of Life. (President's Address).** By E. A. Minchin. (*Journ. Quekett Microscopical Club*, 1911.)

38. **The Transmission of** *Trypanosoma lewisi* **by the Rat-Flea (***Ceratophyllus fasciatus***).** By E. A. Minchin and J. D. Thomson. (*British Medical Journal*, I., 1911.)

39. **The Differentiation of the Mannite-Fermenting Group of** *B. dysenteriæ* **with special reference to Strains isolated from Various Sources in this Country.** By H. de R. Morgan. (*Journal of Hygiene*, Vol. XI., 1911.)

40. **Remarks on the Bionomics of Helminths.** By W. Nicoll. (*British Medical Journal*, I., 1910.)

41. **On the Entozoa of Fishes from the Firth of Clyde.** By W. Nicoll. (*Parasitology*, Vol. III., 1910.)

42. On *Gasterostomum tergestinum*, **Stossich.** By W. Nicoll. (*Annuario del Museo Zoologico della R. Universita di Napoli*, Vol. III., 1910.)

43. **A Cabinet for Holding Small Specimen Bottles.** By W. Nicoll. (*The Museums Journal*, 1911.)

44. **On the Part Played by Flies in the Dispersal of the Eggs of Parasitic Worms.** By W. Nicoll. (*Local Government Board Report*, 1911.)

45. **Two Species of Cysticercoids from the Rat-Flea** (*Ceratophyllus fasciatus*). By W. Nicoll and E. A. Minchin. (*Proc. of the Zoological Society of London*, 1910).

46. **Guinea-Pigs as Chronic Carriers of an Organism belonging to the Food-Poisoning Group.** By R. A. O'Brien. (*Journal of Hygiene*, Vol. X., 1910.)

47. **Studies in the Anaërobic Culture of the Intestinal Micro-Organisms.** By W. J. Penfold. (*British Medical Journal*, II, 1910.)

48. **Variations of the Fermentation Properties of the** *B. typhosus*. By W. J. Penfold. (*British Medical Journal*, II., 1910.)

49. **Variability in the Gas-Forming Power of Intestinal Bacteria.** By W. J. Penfold (*Proc. Royal Society of Medicine*, February, 1911.)

50. **Studies in Bacterial Variation.** By W. J. Penfold. (*Journal of Hygiene*, Vol. XI., 1911.)

51. **Bionomics of Pathogenic Organisms.** By G. F. Petrie. (*British Medical Journal*, II., 1910.)

52. **Report upon the Examination of Rats Collected in Suffolk and Essex for Plague Inspection.** January 16th to February 14th, 1911. By G. F. Petrie and G. H. Macalister. (*Appendix to the Report of the Medical Officer of the Local Government Board*, 1911.)

53. **A Guinea-Pig Epizootic Associated with an Organism of the Food-Poisoning Group, but probably caused by a Filter-Passer.** By G. F. Petrie and R. A. O'Brien. (*Journal of Hygiene*, Vol. X., 1910.)

54. **The Experimental Production of the Carrier-State by Feeding.** By G. F. Petrie and R. A. O'Brien. (*Proc. Royal Society of Medicine*, December, 1910.)

55. **Further Results of the Experimental Treatment of Trypanosomiasis; being a Progress Report to a Committee of the Royal Society.** By H. G. Plimmer, W. B. Fry and H. S. Ranken. (*Proc. Royal Society*, Vol. B., 83, 1910.)

56. **Studies on Ceylon Hæmatozoa.** No. II. **Notes on the Life-Cycle of the** *Hæmogregarina nicoriæ*, **Castellani and Willey.** By MURIEL ROBERTSON. (*Quarterly Journal of Microscopical Science*, Vol. LV., 1910.)

57. **Transmission of Flagellates Living in the Blood of Certain Fresh-Water Fishes.** By MURIEL ROBERTSON. (*Philosophical Transactions, Royal Society*, 1911.)

58. **The Division of the Collar-Cells of** *Clathrina coriacea* **(Montagu): a Contribution to the Theory of the Centrosome and Blepharoplast.** By MURIEL ROBERTSON and E. A. MINCHIN. (*Quarterly Journal of Microscopical Science*, Vol. LV., 1910.)

59. **First Report on Investigations into Plague Vaccines.** By S. ROWLAND. (*Journal of Hygiene*, Vol. X., 1910.)

60. **The Quantitative Estimation of Indole.** By H. SEIDELIN. (*Journal of Hygiene*, Vol. XI., 1911.)

61. **The Action of** *B. lactis aërogenes* **on Glucose and Mannitol.** Part II., **Investigation of the 2: 3 Butanediol and the Acetylmethylcarbinol Formed; the Effect of Free Oxygen on their Production; the Action of** *B. lactis aërogenes* **on Fructose.** By G. S. WALPOLE. (*Proc. Royal Society*, Vol. B., 83, 1911.)

62. **Studies on Avian Hæmoprotozoa.** I. **On Certain Parasites of the Chaffinch** (*Fringilla Cœlebs*) **and the Redpoll** (*Linota rufescens*). By H. M. WOODCOCK. (*Quarterly Journal of Microscopical Science*, Vol. LV., 1910.)

63. **Über die Zusammensetzung des durch Hefepressaft gebildeten Hexosephosphorsaure,** Part II. By W. J. YOUNG. (*Biochemische Zeitschrift*, Bd. 32, 1911.)

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DIPHTHERIA CARRIERS.

Being a Paper read in the Section of Bacteriology at the Annual Meeting of the British Medical Association, London, July, 1910.

By Joseph A. Arkwright, M.D Cantab.,
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Before the specific bacillus was discovered it was recognized clinically that persons who had not themselves suffered from recognizable diphtheria, but who had recently recovered from a sore throat, were liable to give diphtheria to others. The occurrence of the *Bacillus diphtheriae* in the fauces of patients three to fourteen days after the disappearance of the membrane was described by Roux and Yersin in 1888; and Loeffler reported in 1890 a case in which bacilli able to cause the disease, were found in the fauces of a patient three weeks after apparent recovery. Since that time numerous observers have recorded the persistence of the bacillus and its continued virulence for long periods after all local naked-eye symptoms of the disease have passed away. In fact, this phenomenon has become one of the best known facts of bacteriology, which are of practical everyday interest, and at the same time one of the chief bugbears of general practitioners and medical officers of health. Moreover, it is not only during convalescence from diphtheria, and after slight attacks of sore throat, that the bacillus may be present, but also after contact or association with diphtheria patients, both adults and children are liable to harbour the bacillus in their fauces, even when no local or constitutional disorder of any kind has been noticed.

There are thus two classes of healthy carriers of the diphtheria bacillus. First, "convalescents" who have had the disease and have retained the bacillus after apparent return to health; and secondly, "contacts," who have never had the disease at all as far as can be discovered, but who have at some time been in contact with a case of diphtheria or another "contact." To these two groups must be added a third group of carriers, consisting of those persons who are suffering from the disease in a chronic form without constitutional symptoms. These latter are very important as regards the spread of the disease. In some cases the chronic form appears to have existed in a patient for years, though it cannot be stated that no intermission has occurred during that time. For

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instance, one may cite the case of a nursemaid recorded by E. Neisser, who thought it probable that she had had the disease on and off for fourteen years. Cases of membranous or fibrinous rhinitis due to the diphtheria bacillus are best included in this group; some of these cases last for at least two or three years.

The large majority of healthy carriers of both the convalescent and contact classes soon lose the bacilli. Probably about half the convalescents are free by the time that the local exudation on the fauces has disappeared. Welch, Prip, and Glucksam quote large numbers of cases which support this statement.

Welch examined 752 cases, and of these 427 only retained the bacillus after the disappearance of the membrane. One week after disappearance of membrane 47 per cent. of the 427 were still infected; one month after, 2.5 per cent. were still infected.

Prip examined 654 patients in hospital, of whom 345 retained the bacillus after disappearance of membrane: 38 per cent. for ten days from this date; 16.5 per cent. for thirty days from the same date; 2 per cent. for two months; 0.65 per cent. for three months.

Prip also examined 60 discharged patients. One month after discharge 20, or 33 per cent., retained the bacillus; after two months 11, or 18 per cent.; after three months 3, or 10 per cent.; and after twenty-two months 1 person still retained the bacillus.

Glucksam examined 484 convalescents, of whom 267 still had the bacillus in the fauces. Of these 267, 17, or 8 per cent., still retained the bacillus seven days later, and 4, or 1.5 per cent., four weeks later.

Scheller examined 389 convalescents. Of these, 264, or 77 per cent., still retained the bacillus after eleven days; 62, or 18 per per cent., after thirty-one days; 18, or 5 per cent., after sixty-one days; and 8, or 2 per cent., after ninety days.

Drigalski examined infected schools, and found 218 contacts to be carriers. Of these, 8, or 4 per cent., retained the bacillus for over three weeks; and 1, or 0.5 per cent., for over three months.

Tyaden, in 1,338 cases which he examined from the onset or early in the disease, found the bacillus still present after one month in 16.4 per cent., after two months in 0.26 per cent., and after three months in 0.05 per cent.

Graham-Smith collected records of 9,080 cases examined, and calculates that the bacillus is still present after one month in 19 per cent., after two months in 7 2 per cent., after three months in 2 per cent., and over 200 days in 0.02 per cent.

Most of these figures it will be observed do not represent the proportion of cases of diphtheria which retain the bacillus for the various periods of time, but the percentage of convalescents who continue to carry, and as only rather over half the diphtheria patients still retain the bacillus into convalescence, the percentages for the retention of *B. diphtheriae* for the whole number of patients would be about half those given for convalescents.

Taking the figures as a whole it appears that about one-half of the diphtheria patients are free from diphtheria bacilli within two or three days of the disappearance of the membrane, and of the remainder probably 50 per cent. are free in another week or ten days.

At the end of a month from the disappearance of the exudation only 16 to 19 per cent. retain the bacillus, after two months only 5 to 7 per cent., and after three months at most 1 to 2 per cent. These last, the 1 to 2 per cent. who retain the bacillus after three months from convalescence, constitute what may be called the chronic carriers, though the evidence does not point to their being perpetual carriers as in the case of typhoid carriers, for only a very small percentage of healthy carriers carry for six months, as far as is known. Graham-Smith's figures show that only 0.02 per cent. carried for more than two hundred days.

Prip recorded a case of a virulent *B diphtheriae* being still found after more than three hundred days, and of a *B. diphtheriae* as recognized by its morphology only after four years.

On the other hand, cases of chronic diphtheria chiefly constitute the group of carriers who retain the bacillus for an indefinite period.

The persistence of the bacillus in contacts is much the same as in carriers. Probably the contacts do not retain the bacillus for so long (Kober and Glucksam)

I found in one infected school (Arkwright, 1908) 118 carriers out of 551 contacts examined, or 21 per cent.

- Fourteen days later, of 91 examined, 23, or 19.5 per cent., were carriers.
- Twenty-eight days later, of 31 examined, 10, or 8.5 per cent., were carriers.
- Six weeks later, of 11 examined, 8, or 6.8 per cent., were carriers.
- Eight weeks later, of 9 examined, 7, or 6.0 per cent., were carriers.

In another school examined more recently, two swabbings of the whole school (145) showed 10 carriers. All the strains were virulent.

In one case the bacillus persisted till the patient ceased to send swabs—three weeks. One ceased to carry in three weeks. One ceased to carry in six weeks. One ceased after eighty-five days.

The percentage of healthy carriers among those who have recently come into contact with cases of diphtheria varies very much according to circumstances, such as the closeness of contact and the habits and social position of the population examined (cf. Pennington). It has sometimes happened that as many as 20 per cent. of the children in an infected school have been found to harbour *B. diphtheriae*, and Scheller found in several families in which the members were repeatedly examined, that every member became infected sooner or later. On the other hand, in communities where diphtheria has not occurred for some time the percentage of carriers varies from 2.5 to 1 per cent., or in some series of examinations to zero—for example, Ustvedt in certain isolated schools in Norway (86 children), and Scheller. The percentage of virulent *B. diphtheriae* in non-contact communities is very small, say, 0.04 per cent. (Graham-Smith).

Little is known of the cause of persistence of the bacillus. Some observers have pointed out that enlarged tonsils favour prolonged retention of the *B. diphtheriae.* Meikle, who methodically examined a large number of diphtheria convalescents, states that out of 27 persistent carriers 22 had enlarged tonsils. The *B. diphtheriae* has been found in the antrum, sphenoidal and frontal sinuses, and middle ear (Wolff and Councilman, Mallory and Pearce)

Nose.—The figures given above have referred only to observations made by taking swabs from the fauces. The nose has been less frequently examined. Except in cases where there is nasal discharge, the number of carriers in whom the *B. diphtheriae* is confined to the nose is probably small. But Graham-Smith collected records of 5,826 persons examined, amongst whom 2·33 per cent. were found to have *B. diphtheriae* present in the nose only. Pugh found *B. diphtheriae* in the nose of 23 out of 414 scarlet fever patients; 3 of these strains were tested for virulence, and found to be non-virulent. There is greater liability to confuse diphtheria-like bacilli from the nose with *B. diphtheriae*, as various diphtheroids are of more frequent occurrence in the nasal fossae than in the fauces.

Ear.—Graham-Smith isolated 17 strains of *B. diphtheriae* from cases of otorrhoea, and found 11 non-virulent and 6 virulent. The liability to confusion is greater here than in the case of the nose, and the difficulty of isolation is much greater, on account of the saprophytic organisms found in aural discharge.

Treatment of Carriers

The means at our disposal of freeing the throat or nose from the bacillus in the case of carriers are not very efficient. Diphtheria antitoxin given subcutaneously is of no value for this purpose, according to all observers. Different kinds of antiseptic gargles have not given encouraging results when tested methodically; and the use of antibacterial serum locally, though recommended by Louis Martin and also prepared by Wassermann for the purpose, have not been successful in other hands. Vaccination with killed diphtheria bacilli in increasing doses, first tried on 5 carrier-cases by Petruschky, has not, so far as I am aware, been tried in a large number of cases and deserves further trial, but the few recorded cases which I have met have been quite inconclusive. Operative treatment on the tonsils, where these appear to be the seat of the bacilli, seems a hopeful and justifiable procedure. Probably the whole of the tonsillar tissue should be removed as far as possible, as recommended by Pegler. A prophylactic dose of antitoxin should be given before the operation. It has been recommended that the tonsils should be painted with some chemical substance, such as iodine, which will cause a local inflammation and lead to destruction of the bacilli by the inflammatory exudate, and also their extrusion from suppurating tonsillar follicles. In any case antiseptic gargles, etc., should be used frequently with the view of lessening the spread of the bacilli to others.

The nature of the immunity from the disease which the carriers enjoy is obscure, but it is not always complete nor as persistent as the bacilli. Carriers may develop diphtheria after tonsillotomy or operation on the turbinated bones, and, without any known localized wound, may suddenly develop the disease after carrying the bacillus for three or four weeks (Scheller). Prophylactic doses of antitoxin may cause similar temporary immunity with presence of bacilli in the fauces, and later an attack of diphtheria may occur—for example, 21 contacts were injected prophylactically by Peters, of whom 7 developed diphtheria after three weeks.

It is at first sight remarkable that so few cases of diphtheria occur in schools in which, perhaps, 7 per cent. of the children have *B diphtheriae* in their throats

On the other hand, numerous cases are known of the disease resulting from short contact with a healthy carrier or with cases of chronic diphtheria. However, even those in close contact with diphtheria cases often escape the disease, and very frequently only one or two members of a numerous household are attacked with recognizable diphtheria where isolation is not practised.

Ustvedt only observed 3 secondary cases from 98 persons suffering from diphtheria Cases of chronic fibrinous rhinitis give rise to singularly few cases of clinical diphtheria, although virulent *B diphtheriae* can be readily isolated from them. Lack examined 37 cases of fibrinous rhinitis and isolated virulent *B. diphtheriae*, but from none of them—nor, indeed, from 50 cases in all—could he hear on careful inquiry of any case of diphtheria originating. Other observers, however, have recorded instances in which severe cases of diphtheria have resulted from contact with a case of membranous rhinitis. In the case of school carriers, it seems likely that some are far more dangerous than others, for if a school class is swabbed only in part, and therefore probably only a certain number of the carriers are isolated, this partial removal of carriers is often sufficient to stop an epidemic.

The following question arises practically. Taking an otherwise healthy person who is a carrier of *B. diphtheriae*, as shown by the ordinary methods of swabbing serum tubes and examination of a film, is he necessarily a danger to his surroundings unless isolated?

The relative efficiency of different carriers must be taken into consideration, and, further, the possible value of antiseptic gargles in preventing the distribution of the bacilli. This has, as far as I know, never been sufficiently inquired into and tested, but undoubtedly some carriers have living virulent *B. diphtheriae* in their mouths within an hour or two of active antiseptic treatment.

Does a diagnosis of the *B. diphtheriae* by examination of a film from a young serum culture definitely decide the dangerous nature of this bacillus? With the greatest care and experience bacilli resembling virulent *B. diphtheriae* are sometimes found in the fauces or nose which are non-virulent for guinea-pigs and produce no toxin capable of killing a guinea-pig (These bacilli are at the time, therefore, probably non-pathogenic for man.)

What is the nature of these bacilli, and can those who harbour them pass on diphtheria?

These bacilli which resemble *B. diphtheriae* morphologically are not all of the same kind and should be classified in accordance with the scheme of Park and Beebe, advocated by Graham-Smith, namely:

1. Bacilli which do not ferment glucose.
2. Bacilli which do produce acid from glucose, which are non-virulent for guinea-pigs, but resemble virulent *B. diphtheriae* in every other respect.
3. Bacilli which ferment glucose and are virulent for guinea-pigs.

1. Rarely a bacillus of the Hofmann type resembles a virulent *B. diphtheriae* morphologically. When isolated, such a bacillus is easily distinguishable by the fact that it does not ferment glucose.

2. Certain other bacilli occur which closely resemble *B. diphtheriae* morphologically, but have different characters when grown on solid media. I have come across several such in supposed carriers, which when isolated are easily distinguishable from *B. diphtheriae* by the opacity of the growth on agar and by the fact that they ferment cane sugar as well as glucose.

3. Besides the above, strains are met with which resemble the true *B. diphtheriae* in every respect, but which are quite non-pathogenic for guinea-pigs in ordinary doses and produce none or only very small quantities of specific diphtheria toxin.

Graham-Smith has very carefully discussed the question of the nature of these non-virulent *B. diphtheriae*, and he points out that they occur, when a large number of observations are collected, in from 1 to 2 per cent. of all healthy persons not in contact with diphtheria. Graham-Smith's and Cobbett's figures for the occurrence of these non-virulent *B. diphtheriae* among "contacts" do not exceed this proportion of 1 to 2 per cent. Graham-Smith therefore suggests that the non-virulent *B. diphtheriae* is a distinct bacillus and bears no relation to diphtheria.

The percentage of carriers of non-virulent *B. diphtheriae* among healthy persons who are not "contacts" varies considerably, however, according to different observers, and the proportion of "contacts" who harbour non-virulent *B. diphtheriae* has been found to be much higher in some epidemics than in others. Graham-Smith and Cobbett found no evidence of the occurrence of strains of *B. diphtheriae* of intermediate grades of virulence between full virulence and complete absence of virulence for guinea-pigs. In some epidemics of diphtheria, on the other hand, a considerable number of partially virulent strains have been found. Pennington found in an infected school 9 per cent. of carriers, made up of 3 per cent of carriers of non-virulent *B. diphtheriae*, 1 per cent. of carriers of fully virulent *B diphtheriae*, and 5 per cent. of carriers of *B. diphtheriae* of only slight virulence The standard of virulence was, however, not that used by Graham-Smith and Cobbett; but the difference between the three classes seems so marked that it would probably have come out if the method of testing had been different.

In 1908, in the *Journal of Hygiene*, I recorded the results of the investigation of an epidemic of diphtheria in a school. I found a high proportion of non-virulent and of partially virulent strains among the diphtheria bacilli isolated from the carriers. Of 590 boys, 23 per cent. yielded *B diphtheriae*. Twenty strains were isolated and tested for virulence, and if these strains were a fair sample of the whole, then,

7 per cent. of the boys harboured non-virulent strains
6 per cent. of the boys harboured partially virulent strains
7 per cent. of the boys harboured fully virulent strains.

Although very careful observations made by Park and Williams and others have shown that, as a rule, the strain of *B. diphtheriae* isolated from a patient retains its virulence up to the latest date at which it is possible to find it in that patient's fauces, still, sometimes a strain isolated from a case of diphtheria proves to be non-virulent. Lubowski (1900) found, amongst other diphtheria cases, two diphtheria patients from whom he could only isolate non-virulent strains of *B. diphtheriae*, and he found unusually high antitoxin content in the blood of both these patients. Pennington found on examining 25 diphtheria convalescents that 22 yielded virulent strains of *B. diphtheriae* and 2 non-virulent strains five to seven days after the end of their illness. One convalescent yielded at first a virulent strain and later a non-virulent strain.

In the school epidemic mentioned above, the first case of severe sore throat occurred in a boy who had returned to school before I examined the boys. He had suffered from a thin purulent nasal discharge for the greater part of the time since his illness. It seemed highly probable that he was responsible for a large share in the spread of the disease On several occasions, however, the only strain of *B. diphtheriae* which could be isolated was completely non-virulent, although in all other respects exactly resembling typical virulent *B. diphtheriae*. Similar strains were isolated from his fauces and his nose. The evidence for the transformation of true non-virulent *B. diphtheriae* into virulent *B. diphtheriae* or the reverse is scanty and unsatisfactory. I have tried very many methods, but have completely failed to change the one type into the other.

Occasionally, however, a strain on being tested has proved non-virulent, whereas on a former occasion the same strain has been virulent. When this has occurred, the change has not been referable to any special treatment or external condition, and has been permanent.

All the non-virulent strains with which I have worked have been completely non-pathogenic unless injected in massive doses intraperitoneally, and then antitoxin has not had any neutralizing effect when given beforehand or simultaneously.

Although I have not been able to obtain any direct evidence of toxin or toxoid produced by non-virulent strains, still the injection of filtrate from one non-virulent strain into a horse caused a decided rise in the antitoxin content of the horse's serum—from ⅕ to 4 units—and the further injection of filtrate from cultures of another non-virulent strain further raised the antitoxic value of the serum to 25 units per c.cm. (Arkwright, 1910).

It does not seem, therefore, to be definitely settled whether *B. diphtheriae*, which are typical in every way except that they are non-virulent, have any close causal relationship to cases of diphtheria or not Very probably they are less likely to cause diphtheria in man than virulent *B. diphtheriae*, for the circumstances under which they regain their virulence—if they do so—are quite unknown.

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Hypersensibility to pure egg albumin.

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The object of the present communication is to offer evidence in support of the following points: 1) that the phenomenon of hypersensibility can be elicited with pure egg albumin, 2) that the hypersensitizing substance does not, as stated by Victor Vaughan, appear to consist of two portions, the one of which hypersensitises and the other intoxicates, 3) that the reaction product formed by the first injection of albumin is an amboceptor, but is not identical with a free precipitin receptor, 4) that as far as egg albumin is concerned, the doctrine of "anti-anaphylaxis", as propounded by Besredka, needs readjustment; the assumed anti-anaphylaxis being in many instances a complete or partial immunity and 5) that hypersensitization may outlast a definite reaction after the second injection and therefore that the failure to react on the third injection is no evidence of hypersensibility at the time of the second injection.

In view of the interim nature of the report, no attempt will be made to discuss the whole literature of the subject, and for this reason, reference will only be made to those contributions, which deal directly with the subject matter of the report.

Gideon Wells (1908, 1909) has directed his attention to the subject of hypersensibility to pure egg albumin and his accounts largely agree with the results which I have obtained during the past two years. Victor Vaughan (1907, 1909) has experimented with egg white and claims to have obtained results of a far reaching character, and on the basis of these results, Detre stated at the International Medical Congress in Budapest (1909) that he found support for the views which he held on the mechanism of hypersensitization.

Method of preparation of crystalline egg albumin.

The egg albumin used in the experiments was prepared according to the method described by F. G. Hopkins (1899).

White of egg is beaten up into a froth with exactly its own bulk of saturated ammonium sulphate solution. A bulky precipitate containing the globulins etc. separates out and after the fluid has stood overnight, this precipitate is filtered off and the filtrate carefully measured. To the filtrate a 10 % dilution of acetic acid is gradually added from a burette until a well marked precipitate forms. This amount corresponds roughly to incipient acidity of the solution toward litmus. A further cubic centimetre of acid is then added for each 100 ccm of the filtrate, and the fluid is then put aside and stirred from time to time. It has been found advisable to sew this fluid with some crystals of egg albumin, in order to hasten the complete crystallization of all the albumin. From time to time a drop is placed on a slide and examined under the microscope, to determine whether all the precipitate has become crystalline. When the process is finished, the crystals are filtered off through a hardened filter paper. After pressing the precipitate between layers of blotting paper to abstract as much of the fluid as possible, the cake remaining is redissolved in a minimal quantity of water. The protein is the reprecipitated by the addition of a solution of ammonium sulphate until distinct flakes remain permanently in the fluid. To this a few crystals are added and recrystallization takes place.

The crystals pressed free of superfluous fluid may be stored in cake-form or dried in a dessicator and kept as a powder. For immediate use, the crystals are placed in a suitable dialysor and freed from all traces of ammonium sulphate. The last stages of dialysis should be conducted with distilled water. The solution may then be rendered isotonic by the addition of a weighed quantity of sodium chloride and standardized. This may be done by weighing the precipitate gained by heating a measured sample of the solution, or by the determination of the N content by Kjeldahl's method.

The effect of the injection of pure egg albumin.

Guineapigs were given injections of varying amounts of pure egg albumin up to 2 grams both subcutaneously and intraperitoneally, without any toxic symptoms developing. Toxic symptoms and death however were produced when a second dose was given after a lapse of at least ten days.

The hypersensitizing dose: It was found that any dose from 0,0001 up to 2 grams at the first injection sufficed to produce hypersensibility. For practical purposes, however, it appears that a dose varying between 0,04 and 0,08 g. yields the best results. When pretreated in this way, every guineapig was found to be hypersensitive.

The interval: After 10 days the animals were found to be hypersensitive, but death or very severe symptoms, including paralysis, convulsions and coma always followed if the second injection was carried out on the 14th day or later. It has been found that the hypersensibility can last for a considerable period, e. g. it was present on the 124th day, on the 159th day and on the 240th day in several instances. Rosenau and Anderson (1908) have placed on record that hypersensibility to horse serum was in one case still present after 732 days.

The intoxicating dose: Death resulted when egg albumin in solution was injected intraperitoneally in doses of from 0,001 to 2 g. The smallest dose which invariably killed the guineapig was found to be 0,04 g. 0,0004 g. frequently produced paralysis, from which recovery resulted. In the majority of the experiments, 0,06 to 0,08 g. was used for the second injection, this dose being rapid and striking in its action.

Intravenous, intra-cranial and intra-cardiac injections were employed in a certain nunber of cases. The dose sufficient to produce intoxication and death by these methods was, as would be expected, lower than that required when the intraperitoneal method was used, but there were objections to the habitual employment of each of the methods enumerated, save the intracardiac. In the case of intracranial injections, even when the total quantity of fluid injected was kept down to 0,3 ccm, death occasionally ensued, and it was found that severe paralytic disturbances resulted at times, when physiological salt solution was injected in the same dose and by the same method.

Passive hypersensitization in guineapigs: The serum of hypersensitive guineapigs proved to possess irregular and little marked power of inducing hypersensibility in normal guineapigs. Among a considerable number of sera tested, only a few possessed this capability, and each sample possessed a different hypersensitizing power. Reference to these experiments will be made under the heading of the reaction product of egg albumin. On the other hand, it was found that the serum of rabbits, which had received injections of pure egg albumin possessed this power of imparting hypersensibility to normal guineapigs in a marked manner. The mode of preparing both guineapigs and rabbits for this purpose consisted in giving a number of injections subcutaneously, intravenously or intraperitoneally. For guineapigs, subcutaneous injections appeared to be preferable, and for rabbits the intraperitoneal injections proved advantageous. Hypersensibility to egg albumin in rabbits, like hypersensibility to other proteid substances, differs from that in guineapigs, in that the only symptoms seen are sudden pallor, associated with some panting, dyspnoea and signs of uneasiness. It is therefore possible to repeat the injections past the 10th day. If the injections are repeated in guineapigs, it is necessary to anaesthetize the animal after the first injection in the hypersensitive period, until all symptoms have passed off, and then to be careful in giving subsequent injections not to pass on to the injection of large amounts, unless the guineapig has proved itself capable of tolerating small doses well.

The test of passively induced hypersensibility was carried out by giving varying quantities of the serum of the pretreated animal either intraperitoneally or intracardiacally and after the lapse of 24 hours injecting a toxic dose of egg albumin.

Quantitative estimations of the power of a hypersensitizing serum: In order to decide whether the power of hypersensitizing by means of serum could be measured quantitatively, the serum of pretreated rabbits was used in the first place. From the accompanying table, it will be seen that while this method may be utilised for the purpose of roughly comparing sera with regard to their power of inducing hypersensibility, the individual variations in the guineapigs are factors which render the test but little delicate.

Table I.

Rabbit	Serum of rabbit dose	Method of application	Interval in hours	Dose of egg alb.	Result.
B	2 ccm	ip.	24	0,09 g.	†
B	2 „	„	24	0,09 „	0
B	3 „	„	24	0,09 „	†
B	3 „	„	24	0,09 „	S
C	0,5 „	„	24	0,06 „	S
C	0,5 „	„	24	0,06 „	0
C	0,2 „	„	24	0,06 „	S
C	0,2 „	„	24	0,06 „	0
C	0,05 „	„	24	0,06 „	0
D	0,05 to 0,5 ccm	„	24	0,06 „	0
D_2	0,02 to 0,5 ccm	„	24	0,06 „	0
D_3	0,45 „	„	24	0,08 „	S
D_3	0,2 „	„	24	0,08 „	S
D_3	0,1 „	„	24	0,08 „	0
D_2	0,05 „	„	24	0,08 „	S
D_4	1 „	„	48	0,06 „	†
D_4	0,5 „	„	48	0,06 „	†
D_4	0,1 „	„	48	0,06 „	S
F	0,5 „	„	24	0,06 „	†
F	0,2 to 0,05 ccm	„	24	0,06 „	0
G	0,5 „	„	24	0,06 „	S (late)
G	0,2 „	„	24	0,06 „	S (late)
G	0,1 „	„	24	0,06 „	S
G	0,05 „	„	24	0,06 „	0
H 1)	2 „	„	24	0,09 „	0

† = died., S = symptoms, including paralysis, 0 = either no symptoms or symptoms without paralysis, ip. = intraperitoneal.

D = after 5 injections, D_3 = after 8 injections,
D_2 = „ 6 „ D_4 = „ 10 „

Experiments with mixtures of the serum of pretreated animals and egg albumin were also carried out but for the sake of convenience, these experiments will be considered later.

Attempts to split up the protein molecule into constituents.

The work of Victor Vaughan (1907, 1909) indicated that egg white could be split up into two component parts, one of which acted as a hypersensitizer and the other as an intoxicator. These fractions he called haptophore and toxophore, respectively. Crystalline egg albumin was treated in exactly

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1) This rabbit had only received 3 injections.

the same manner as the egg white in Vaughan's experiments. The details were followed closely with special care. In the first place, it was found that the fraction which was insoluble in alcohol (cf. Vaughan's haptophore) was toxic to normal guineapigs in doses of 0,1 g. The symptoms produced were twitching of the whole body, paresis of the hind extremities, convulsions, dyspnoea. At times death occurred suddenly. 0,05 g. or less did not appear to produce toxic symptoms. The fraction which was soluble in alcohol (analogous to Vaughan's toxophore) did not produce symptoms in doses up to 0,15 g. Guineapigs were pretreated with the first fraction in doses varying between 0,0005 g. and 0,05 g. On being tested on the 14th day or later, no toxic symptoms were produced, when either whole egg albumin or either fraction were employed. It was further noticed that guineapigs which had received a first dose of the first fraction, became somewhat more resistant to a second dose of the same substance.

Guineapigs were also pretreated with the alcohol soluble fraction in doses varying between 0,00039 and 0,146 g. A very slight degree of hypersensitization to whole egg albumin and to itself but not to the fraction insoluble in alcohol resulted. In no case, did the second injection produce even severe symptoms.

Guineapigs which had been sensitised with egg albumin were tested with the two fractions. In full doses, the fraction corresponding to Vaughan's toxophore produced slight symptoms. In one case a guineapig was given three injections, the first of egg albumin, the second of the alcohol soluble fraction on the 19th day and the third of the same substance on the 89th day. Some convulsive movements followed the second injection, and death followed the third. Several other guineapigs received repeated injections of both fractions, but with entirely negative results.

These experiments show that when pure egg albumin is employed, the portion corresponding to Vaughan's haptophore fraction is incapable of hypersensitizing normal guineapigs to itself, to the other fraction or to whole egg albumin. On the other hand the portion corresponding to toxophore is capable to a very slight extent of hypersensitizing, in spite of Vaughan's statements and under certain circumstances may intoxicate when applied as a third injection. From these conclusions, the natural deduction would be that the treatment of egg albumin with alcoholic solutions of caustic soda destroys the hypersensitizing and intoxicating action to a large extent.

The hypersensibility to pure egg albumin was not interfered with by an interposed injection of either fraction of the albumin. On subsequent testing with egg albumin, the guineapigs were found to be hypersensitive.

Other modes of breaking down the protein molecule. The work of Gideon Wells with regard to the digestion of egg albumin with proteolytic ferments, as well as the experiments with heat were to a large extent confirmed and show that the protein molecule as such acts in the phenomenon and that all processes which tend to break down the molecule, interfere to a proportionate extent with the hypersensitizing and intoxicating action of the albumin.

The reaction product of egg albumin.

Experiments were conducted with a view of ascertaining whether the reaction product, produced by the first injection, is capable of rendering the albumin toxic by mixture in vitro. Reference should be made in this place to the highly interesting experiments of Friedberger (1909, 1910) who showed that when dealing with serum, a mixture of the test serum and the serum of the pretreated animal produces a precipitate, which when abstracted with normal guineapig's serum, yields a toxic product. He calls this soluble toxin "anaphylatoxin", and believes that the symptom complex produced by this substance is identical with hypersensitization poisoning. In his previous articles, Friedberger set up the thesis that the reaction product which is formed by the first injection and which produces the changes in the so called passive hypersensitization experiments, is probably a free precipitin receptor [1]. Biedl and Kraus (1909) have also brought some interesting information to light, with regard to the reaction product. Attention however must be called to the fact that all these investigators have worked with serum almost exclusively, and as will be explained in a subsequent communication, there is every reason to believe that the hypersensibility produced by serum is not a simple increase of toxicity of one protein but is a compound alteration of the toxicity of a number of organic substances and that many of the phenomena which have been observed may find their explanation in this fact. Further a number of authors constantly employ the term „anaphylaxis“ in the sense of a definite toxicological complex. It is probable that this use of the term, which has many other objections, may hamper the investigator, since there is at present no proof that the phenomenon is more than the result of the toxic action of a newly formed body, which owes its origin to the reaction of the body to the introduction of a substance which in itself is harmless to the organism. It would therefore be probable that the symptoms and pathological changes of what is known as the phenomenon of hypersensibility, would vary according to the nature of the substance introduced at the first injection, although the future must prove whether the organism is capable of producing one and the same reaction product in response to the introduction of two different proteins. Biedl and Kraus found that a mixture of the serum of hypersensitive guineapigs and the serum used in the preparation of the same acquired toxic properties which were not possessed by either components before mixture and that this toxic property could be increased by passing the mixture through the body of a normal guineapig. Richet (1907) and Friedemann (1909) have also met with an increase of toxicity of the test serum by mixture with the serum of the prepared animal.

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[1] Anm. bei der Korrektur. Friedberger vertritt die Ansicht, daß das Reaktionsprodukt bei Einverleibung von Eiweiß — Antieiweiß — einen einheitlichen Körper darstellt. Die Anaphylaxie hervorrufende Eigenschaft ist viel empfindlicher als die präzipitierende. Doerr identifiziert das anaphylaktische Reaktionsprodukt mit dem Präzipitin.

The majority of the experiments undertaken in regard to this point which were conducted with the serum of guineapigs proved negative but as has already been shown, the serum of hypersensitive guineapigs possesses little power of hypersensitizing normal guineapigs and that this power, when present, is variable. From time to time, however, positive results were obtained. Tests were then applied with the serum of pretreated rabbits with the result that less irregular results were obtained. It was, however, found that save on a few occasions, the simple mixture of egg albumin and rabbit's serum did not suffice to produce a fluid, which called forth symptoms similar to those seen in the shock of egg albumin hypersensibility. Regular results were obtained when an appropriate quantity of normal guineapig's serum was added in vitro to the egg albumin serum mixture. Working with the serum of Rabbit D after the 10th injection, it was found that when the proportion of normal guineapig's serum (containing complement) to the rabbit's serum was as 3:1, the binding of the reaction product in the serum with the protein took place readily. The dose of rabbit's serum chosen for these experiments was that which had proved sufficient to produce passive hypersensibility, or larger. With regard to the quantity of egg albumin, it was considered that an excess up to a reasonable limit would not interfere with the result, and therefore 6 cg. were used, while in a few experiments, half of this quantity was used. Thus, when 1 ccm of rabbit's serum and 1 ccm of normal guineapig's serum were mixed with varying amounts of egg albumin, up to 13 cg., paralysis and death took place at times, while in other instances no symptoms were produced. On the other hand, when 2 ccm of normal guineapig's serum and 0,68 ccm of rabbit's serum were mixed with 0,035 g. of egg albumin, or 2,75 ccm of normal guineapig's serum and 1,25 ccm of rabbit's serum were mixed with doses of egg albumin varying between 3 and 6 cg., paralysis and death invariably followed immediately. The injections were made intracardially. In each instance, inactivated rabbit's serum was used for these experiments, and it was further noted that the serum of Rabbit D, lost very little power after having been kept for several weeks.

The reaction product an amboceptor.

These experiments were preceded by experiments undertaken to determine whether the substance present in the rabbit's serum was of the nature of an amboceptor. The following table demonstrates clearly that the serum of a rabbit pretreated with egg albumin contains a new product, which is only capable of deviating complement in the presence of egg albumin, and yet which is not identical with precipitin. The latter point can be made clear with a substance like pure egg albumin, which does not readily yield a precipitin and which, when it does, only yields a precipitin of very low value. Rabbit D was first treated by injections of egg albumin dissolved in distilled water, and like the other rabbits, no trace of precipitin developed. It was then given injections of solutions of egg albumin in 0,85 % sodium chloride to which sodium carbonate was added until the free alkalinity corresponded to n/50 sodium carbonate (Hunter). The serum obtained after the 10th injection gave a precipitate in a dilution of 1:20, and a faint cloud in 1:50. In no other serum was any precipitate seen in any dilution. For the sake of ready comparison, a table is set up showing the comparative hypersensitizing, precipitating and deviating values of the various sera.

Table II.

Serum	Hypersensitises in doses of	Precipitin reaction, in dilution of	Deviates 0,1 complement in doses of
Rabbit C	> 0,5 ccm	no reaction	1,0 (a)
Rabbit D_4	< 0,1 > 0,05	1:20 floculent pp. 1:50 slight cloud	0,1
Gnpg. 71/276	3,5 neg.	no reaction	0
„ 69/30	3,5 „	„ „	0
„ 71/246	3,0 „	„ „	0
„ 84/30	3,0 „	„ „	2,0 (a)
„ 39/30	3,0	„ „	1,0 (a)

(a) = not quite complete; neg. = no distinct hypersensibility obtained with given doses.

Table II has been compiled to show the relative power of various sera of hypersensitizing, precipitating and deviating complement. Doerr found a parallelism between the precipitin content of a serum and its power of hypersensitizing. With regard to the sera of animals pretreated with egg albumin, it can be shown that whereas the serum of Rabbit D was the best hypersensitizing serum of the series, it was at the same time the only one which produced any appreciable precipitate with egg albumin in vitro. This fact may be regarded as evidence in favour of Doerr's contention, but the failure of all the other sera to yield any precipitation must also be taken into consideration. It will be seen that the power of deviating complement of Serum D was nearly ten times as strong as that of serum C, and the precipitating power must have been very much more than 20 times as strong, since D produced a cloud in a dilution of 1 : 50, and C did not even produce this effect in any dilution put up. Rabbit D was pretreated with alkaline solutions of egg albumin while in the case of rabbit C the albumin solution employed was slightly acid. There was no precipitin formation in any of the guineapigs. Further experiments are required before any conclusion can be arrived at as to a parallelism between the sensitizing power and the precipitin content of serum in the case of animals pretreated with egg albumin. On the other hand, the parallelism existing between the hypersensitizing power and the deviation in the positive sera is a very striking one[1]).

From table III, it will be seen that serum D in doses of 1 c. c. together with 1 mg of albumin, deviated at least 4 times the dissolving dose of complement required for the system employed, while 0,1 c. c. together with 0,1 mg was also capable of deviating nearly 4 times the dissolving dose of complement. In the first instance, the series was not completed, and it remains doubtful whether it would have been possible to have found the maximum amount of complement, which such a large quantity of ambozeptor as 1 c. c. could claim for itself. In the second instance, it appears that nearly four times its own bulk was deflected from the haemolytic system. This proportion of between three and four times the quantity of complement to amboceptor in serum D was found experimentally to be an optimum for the experiments of rendering the egg albumin solution toxic at the first injection, as stated above.

An apparently contradictory result was obtained in the investigation of the complement content of the sera of hypersensitized guineapigs. In a number of experiments, it was found that there is no ascertainable disappearance of complement after the shock. On the other hand, disappearance of complement has been found by Friedberger and others in connexion with serum hypersensibility. Table III however

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[1]) Anm. bei der Korrektur. Es bedarf, meiner Ansicht nach, weiterer Beweise, ehe man die Möglichkeit von zwei verschiedenen Eiweißantikörpern definitiv ausschließen kann.

Table III.

The following table shows the deviating power of the sera.

A single completely dissolving dose of haemolytic amboceptor for 0,1 ccm of complement and 1 ccm of a 5 % suspension of red blood corpuscles (ox) were used in each case.

	Complement	Serum of rabbit or guineapig	Egg albumin	Result
Rabbit D_4	0,1	1 ccm	0,001 g.	c. d.
	0,2	1 „	0,001 „	„
	0,3	1 „	0,001 „	„
	0,4	1 „	0,001 „	„
	0,1	0,5 „	0,001 „	„
	0,1	0,3 „	0,001 „	„
	0,1	0,1 „	0,001 „	„
	0,1	1 „	0,0001 „	„
	0,1	0,5 „	0,0001 „	„
	0,1	0,1 „	0,0001 „	„
	0,1	1 „	0,00001 „	n. c. d.
	0,1	0,5 „	0,00001 „	p. d.
	0,1	0,1 „	0,00001 „	p. d.
Controls	0,1	2 „	—	p. d.
	0,1	1 „	—	c. h.
	0,1	0,6 „	—	c. h.
	0,1	—	0,002 g.	c. h.
Rabbit D_4	0,1	0,1 ccm	0,0001 „	c. d.
	0,2	0,1 „	0,0001 „	c. d.
	0,3	0,1 „	0,0001 „	c. d.
	0,4	0,1 „	0,0001 „	n. c. d.
	0,5	0,1 „	0,0001 „	p. d.
	0,7	0,1 „	0,0001 „	c. h.
Rabbit C	0,1	1 „	0,0001 „	n. c. d.
	0,1	0,5 „	0,0001 „	p. d.
	0,1	0,1 „	0,0001 „	c. h.
Controls.	0,1	2 „	—	c. h.
Gnpg. 71/276	0,1	1 „	0,0001 g.	c. h.
	0,1	0,5 „	0,0001 „	„
	0,1	0,1 „	0,0001 „	„
Gnpg. 69/30	0,1	1 „	0,0001 „	„
	0,1	0,5 „	0,0001 „	„
	0,1	0,1 „	0,0001 „	„
Gnpg. 71/246	0,1	1 „	0,0001 „	„
	0,1	0,5 „	0,0001 „	„
	0,1	0,1 „	0,0001 „	„
Gnpg. 84/30	0,1	2 „	0,0001 „	n. c. d.
	0,1	1 „	0,0001 „	p. d.
	0,1	0,5 „	0,0001 „	p. h.
	0,1	0,1 „	0,0001 „	c. h.
Gnpg. 39/30	0,1	2 „	0,0001 „	n. c. d.
	0,1	1 „	0,0001 „	n. c. d.
	0,1	0,5 „	0,0001 „	p. h.
	0,1	0,1 „	0,0001 „	c. h.

Controls were put up with each series and showed a complete haemolysis. c. d. = complete deviation; n. c. d. = nearly complete deviatio; p. d. = partial deviation; c. h. = complete haemolysis; p. h. = partial haemolysis.

suggests a plausible explanation for the results obtained with egg albumin. The quantity of complement required to „cover“ a lethal dose of egg albumin is probably so small, that recognisable quantities are not withdrawn from the serum. Further examination of this point is, however, required.

Refractory behaviour to second and subsequent injections.

According to the doctrine of Besredka and others (1907) the refractory behaviour of a guineapig to a third injection is due to a definite anti-anaphylaxis, produced by the second injection. It is unnecessary for the present to discuss the theoretical aspect of the anti-anaphylactic phenomena, or indeed the interpretation of the whole process as given by Besredka, since the object of the present communication is to bring forward evidence of the behaviour of a pure protein when injected repeatedly into the animal body. But it is necessary to point out that a refractory period does not follow in accordance with the rules set up by Besredka and those who follow this investigator's lines of argument. From the first, it became quite clear that it was useless to endeavour to produce a refractory behaviour by injecting first a small dose and then a large one within 24 hours of the first, or first a large one and then a small one, for this purpose. It was found that hypersensitization followed under all conditions within 10 to 14 days of the first dose, no matter how many doses were interposed. A few examples will suffice to demonstrate this. These cases are again set forth in Table IV. Guineapigs received 9 cg. of egg albumin both on the first and on the second day; on being tested with 10 cg. on the 19th day, all showed typical hypersensitization. The hypersensitization followed when 10 cg. were followed by 1 cg. or when 1 cg. was followed by 10 on the following day, in the usual course. At times, after the guineapig had been kept alive by aether anaesthesia at the second injection, subsequent injections had no effect at all, no matter what spacing was chosen (see No. 27 of the Table IV). A similar experiment is found in No. 22, although the symptoms were not stopped by anaesthesia, but by another means, namely by adsorption of the albumin by the brain of a hypersensitive guineapig.

Table

† = died. S = paralysis and convulsions. sl S = convulsions. 0 = less marked sym-
rabbit. x = albumin which had been

No.	Dose of egg albumin	Result	Interval between 1st and 2nd Injection days	Dose of egg albumin g.	Result	Interval between 1st and 3rd injection days	Dose of egg albumin g.	Result	Interval between 1st and 4th injection days	Dose of egg albumin g.	Result
1	0,092 g	0	2	0,092	0	19	0,1	†	.	.	.
2	0,092 ,,	0	2	0,092	0	19	0,1	†	.	.	.
3	0,092 ,,	0	2	0,092	0	19	0,1	†	.	.	.
4	0,092 ,,	0	2	0,092	0	19	0,1	S	.	.	.
5	0.092 ,,	0	2	0,092	0	19	0,1	†	.	.	.
6	0,092 ,,	0	2	0,092	0	19	0,1	†	.	.	.
7	0,1 ,,	0	2	0,01	0	24	0,092	†	.	.	.
8	0,1 ,,	0	2	0,01	0	24	0,092	†	.	.	.
9	0,01 ,,	0	2	0,1	0	24	0,092	†	.	.	.
10	0,01 ,,	0	2	0,1	0	24	0,092	†	.	.	.
11	0,13 ,,	0	3	0,16	0	24	0,092	0	92	0,0195	†
12	0,13 ,,	0	3	0,13	0	15	0,13	†	.	.	.
13	0,13 ,,	0	3	0,13	0	15	0,009	S	18	0,0092	0
14	0,023 ,,	0	4	0,023	0	8	0,023	0	13	0,023	S 1)
15	0,023 ,,	0	4	0,023	0	8	0,023	0	13	0,023	†
16	0,023 ,,	0	4	0,023	0	8	0,023	0	13	0,023	†
17	0,13 ,,	0	5	0,2	0	24	0,092	†	.	.	.
18	0,023 ,,	0	7	0,046	0	14	0,092	†	.	.	.
19	0,023 ,,	0	7	0,046	0	17	0,009	†	.	.	.
20	0,023 ,,	0	7	0,046	0	17	0,009	S	.	.	.
21	0,13 ,,	0	7	0,2	0	24	0,092	S	72	0,092	†
22	0,13 ,,	0	14	0,09	S	63	0,09	0	87	0,0195	0
23	0,001 ,,	0	14	0,092	0	34	0,092	†	.	.	.
24	0,00001 ,,	0	14	0,092	0	34	0,092	†	.	.	.
25	0,092 ,,	0	16	0,092	S	51	0,138	†	.	.	.
26	0,004 ,,	0	23	0,0125	sl S	65	0,00046	0	93	0,13	0
27	0,138 ,,	0	29	0,25	S 1)	63	0,13	0	124	0,092	0
28	0,13 g (Glob.)	0	31	0,134	S	32	0,13	0	.	.	.
29	0,13 ,,	0	66	0,023	0	84	0,018	0	121	0,096	†
30	0,09 ,,	0	76	0,09 partially digested	0	111	0,09	†	.	.	.
31	0,09 ,,	0	76	0,09	0	111	0,09	†	.	.	.
32	0,09 ,,	0	76	0,09	0	111	0,09	†	.	.	.
33	0,13 ,,	0	91	0,092	0	124	0,092	†	.	.	.
34	5 c. c. s. h. g.	0	3	0,13	S	25	0,0195	†	.	.	.
35	3 ,, ,, item	0	3	0,13	S	25	0,0195	†	.	.	.
36	3 ,, ,, ,,	0	3	0,13	0	25	0,0195	0	.	.	.
37	10 ,, ,, ,,	0	2	0,092	sl S	37	0,0092	†	.	.	.
38	10 ,, ,, ,,	0	2	0,092	sl S	37	0,046	S	.	.	.
39	0,08—5 c. c.	0	29	0,00092	S	107	0,13	†	.	.	.
40	0,03—3 ,, ,,	S	23	0,0046	S	138	0,065	0	.	.	.
41	0,001 g	0	12	0,092	0	31	0,092	†	.	.	.
42	0,00001 ,,	0	12	0,092	0	31	0,092	†	.	.	.
43	1 c. c. s. h. g.	0	2	0,08	0	27	0,00046	0	28	0,036	0
44	1 ,, ,, item	0	4	0,08	0	27	0,00046	0	28	0,09	0

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1) Aether anaesthesia.

IV.

ptoms. s. h. g. = serum of hypersensitive guineapig. s. h. r. = serum of hypersensitive artificially altered by freezing.

Interval between 1st and 5th injection days	Dose of egg albumin g.	Result	Interval between 1st and 6th injection days	Dose of egg albumin g	Result	Interval between 1st and 7th injection days	Dose of egg albumin g	Result	Interval between 1st and 8th injection days	Dose of egg albumin g.	Result
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84	0,092	0	131	0,092	†	.	.	.	.	.	.
83	0,023	0	130	0,092	S (late)	198	0,13	0	230	0,065	0
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111	0,092	0	.	.	.	.	.	.	.	.	.
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159	0,92	†	.	.	.	.	.	.	.	.	.
146	0,019	0	170	0,092	0	.	.	.	.	.	.
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No.	Dose of egg albumin	Result	Interval between 1st and 2nd injection days	Dose of egg albumin g.	Result	Interval between 1st and 3rd injection days	Dose of egg albumin g.	Result	Interval between 1st and 4th injection days	Dose of egg albumin g.	Result
45	1 c. c. s. h. g.	0	12	0,092	0	27	0,00092	0	28	0,036 x	0
46	1 ,, ,, item	0	3	0,08	0	27	0,00092	0	28	0,048 x	0
47	3 ,, ,, ,,	0	4	0,08	0	27	0,00184	0	28	0,048 x	0
48	3 ,, ,, ,,	0	8	0,092	0	27	0,0046	S	28	0,06 x	0
49	3 ,, ,, ,,	0	3	0,08	0	27	0,0018	0	28	0,06 x	0
50	5 ,, ,, ,,	0	4	0,08	0	27	0,0046	S	28	0,09	?
51	5 ,, ,, ,,	0	8	0,092	0	27	0,0096	0	28	0,046	0
52	6 ,, ,, ,,	0	3	0,08	0	27	0,0096	S	28	0,046	0
53	0,0001 g	0	16	0,08	S	98	0,065	S	.	.	.
54	0,0001 ,,	0	16	0,08	S	98	0,065	†	.	.	.
55	0,0004 ,,	0	16	0,09	0	98	0,065	†	.	.	.
56	2 c. c. s. h. r.	0	1	0,09	0	87	0,06	†	.	.	.
57	2 ,, ,, item	0	1	0,09	0	80	0,13	0	87	0,065	sl S
58	0,5 ,, ,, ,,	0	1	0,06	S	69	0,065	†	.	.	.
59	0,2 ,, ,, ,,	0	1	0,06	S	69	0,065	sl S	.	.	.
60	0,1 ,, ,, ,,	0	1	0,06	0	69	0,065	S	.	.	.
61	0,05 ,, ,, ,,	0	1	0,06	0	69	0,065	†	.	.	.
62	0,5 ,, ,, ,,	0	1	0,06	0	69	0,065	0	.	.	.
63	0,1 ,, ,, ,,	0	1	0,06	0	69	0,065	†	.	.	.
64	0,2 ,, ,, ,,	0	1	0,06	sl S	69	0,065	†	.	.	.
65	0,1 ,, ,, ,,	0	1	0,06	0	69	0,065	†	.	.	.
66	0,05 ,, ,, ,,	0	1	0,06	0	69	0,065	†	.	.	.
67	0,1 ,, ,, ,,	0	1	0,06	S	69	0,065	†	.	.	.
68	0,5 ,, ,, ,,	0	1	0,065	0	$1^1/_2$	0,129	S	46	0,065	†
69	0,5 ,, ,, ,,	0	1	0,065	0	$1^1/_2$	0,129	S	46	0,065	†
70	0,05 ,, ,, ,,	0	1	0,065	0	32	0,129	†	.	.	.
71	0,45 ,, ,, ,,	0	1	0,08	S	32	0,065	0	.	.	.
72	0,2 ,, ,, ,,	0	1	0,08	S	32	0,065	0	.	.	.
73	0,1 ,, ,, ,,	0	1	0,08	S	32	0,065	S	.	.	.
74	0,05 ,, ,, ,,	0	1	0,08	S	32	0,065	S	.	.	.
75	0,1 ,, ,, ,,	0	1	0,065	S	12	0,065	†	.	.	.
76	1 ,, ,, ,,	0	1	0,065	0	13	0,065	S	.	.	.
77	0,1 ,, ,, ,,	0	1	0,065	0	13	0,065	sl S	.	.	.

It will be seen from the table that in a number of instances the occurrence of symptoms after the second injection did not interfere with the hypersensibility and that death or severe symptoms resulted when the egg albumin was injected after a sufficient interval for a third time (vide Nos 25, 34, 35, 37, 38, 39, 53, 54, 58, 59, 64, 67, 73, 74, 75). A close consideration of the table will reveal that while the hypersensibility is not necessarily destroyed by the second dose productive of symptoms, this depends to some extent on the manner of preparation. It must be pointed out in this place,

Interval between 1st and 5th injection days	Dose of egg albumin g.	Result	Interval between 1st and 6th injection days	Dose of egg albumin g.	Result	Interval between 1st and 7th injection days	Dose of egg albumin g.	Result	Interval between 1st and 8th injection days	Dose of egg albumin g.	Result
87	0,13	†	.	.	.	.	.	.	.	.	.
119	0,065	†	.	.	.	.	.	.	.	.	.
119	0,065	†	.	.	.	.	.	.	.	.	.
(died after 12 hours)			.	.	.	.	.	.	.	.	.
119	0,065	†	.	.	.	.	.	.	.	.	.
119	0,065	†	.	.	.	.	.	.	.	.	.
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that by symptoms is meant paralysis, convulsions and more or less complete unconsciousness. By slight symptoms is meant either convulsions without paralysis or dyspnoea, paresis and inability of sitting or standing. No less marked symptoms were regarded as evidence of hypersensibility, although it could be shown that intraperitoneal injections frequently yielded a negative result, while intracardiac injections caused paralysis or death. It would seem that the sudden fall in temperature during the shock might be a good indication of hypersensibility, as suggested by H. Pfeiffer and S. Mita

(1909), but unless confirmed by some other symptoms, a mere fall of temperature must not be regarded as conclusive proof of the hypersensibility, and further when distinct symptoms of hypersensibility to the special protein employed are seen, the absence of a fall in temperature cannot be regarded as of any diagnostic importance. When the preparatory dose of serum was a large one, especially in the case of rabbits, the reaction process appeared to be somewhat complicated (vide Exp. 71 and 72). When the dose of hypersensitizing serum was unnecessarily large and presumably contained an excess of amboceptor, the hypersensibility in the guineapig was removed by the test injection of egg albumin 24 hours later. Smaller doses (Exp. 73 and 74) of the same hypersensitizing serum produced hypersensibility which at the test injection did not differ ascertainably from that produced by the larger dose, but which did differ from it in that there was no subsequent inhibition and the animal remained hypersensitive to a subsequent injection of egg albumin. A similar inhibition was seen in those cases in which the first injection of serum failed to induce a condition of hypersensibility (vide Nos 36, 43, 44, 45, 46, 47, 49, 51, 57, 62). In each of these cases if the attempt to hypersensitive had not exercised any effect, at all, the test dose of albumin would have rendered the guineapig hypersensitive to a subsequent dose applied 10 or more days later, but this did not occur.

The series 71, 72, 73, 74 illustrates this point. The amount of serum containing amboceptor sufficient to produce hypersensibility was 0,05 (in other experiments with this serum, all the guineapigs save one, which received 0,1 ccm of the rabbit's serum in question died in response to an injection of egg albumin 24 hours later; the guineapigs which were pretreated with 0,05 ccm all recovered after very severe symptoms from the test injection). From the deviation of complement experiments with this serum, it would appear that the amount of egg albumin which the quantities of rabbit's serum employed (e. g. 0,45, 0.2, 0,1 and 0,05 ccm) could combine with is much smaller than the amount of egg albumin injected at the test injection. Allowing that all the albumin would not come into contact with the amboceptor in vivo as it does in vitro, it follows that in each case there must be albumin which is no used in forming the intoxicating substance. When the amboceptor injected is in excess of the amount required to produce a lethal dose of the intoxicating substance, the excess of the combination may be absorbed and give rise to a further antibody — an anti-amboceptor-albumin. In such a case (71, 72) the animal would be immune to a subsequent injection of egg albumin. On the other hand, if all the amboceptor is used up in the poisoning, the excess of egg albumin is free to produce a fresh hypersensitization which is evidenced when a further injection of the albumin is applied at a later date (73, 74). The same mechanism would apply when the amount of amboceptor given at the preparatory injection is insufficient to produce hypersensibility.

It must be born in mind that the amount of amboceptor present in the serum of hypersensitized guineapigs is always small as compared with the amount present in the serum of pretreated rabbits.

The immunity produced by the absorption of the supposed toxic compound (amboceptor-egg albumin) may be real or permanent, as in Nos 14, 22 and 27 or may be merely temporary, as in Nos 13, 26, 45, 47, 49, 51 and 52. In the latter case, it must be assumed that the quantity of the combination is small, so that a temporary inhibition of the hypersensitization is produced rather than a definite immunity.

In the foregoing, I have purposely avoided employing terms suggested by other investigators, such as "apotoxin" (Richet) since the explanations offered are only intended to serve as a working hypothesis and do not in any way claim to be a true solution of the mechanism of hypersensibility.

In order to obtain a further insight into the nature of the reaction product of egg albumin, some experiments were undertaken in which the brain instead of the serum of the pretreated animal was used. The reason why the brain was chosen lay in the fact that Besredka and others have found that a second injection of a protein acts much more violently when applied directly into the brain. Similarly, when a protein is injected upwards through the carotid artery, much smaller doses are, I find, required to produce death, than when the protein is applied by other methods. It must be pointed out that the experiments with brain substance and egg albumin need extension in many directions, and that at present, the utilization of the results is undertaken with reserve. The following Table (V) shows two sets of experiments. In the first series, certain mixtures were injected intraperitoneally into sensitized guineapigs, while in the second, normal guineapigs were employed. The mixtures included 1) brain substance of hypersensitive guineapigs, egg albumin and normal

Tabelle V.

No.	Intervall between hypersensiting and testing injections (days.)	Hours of contact	Amount of brain of hypersensitive guineapig	Amount of egg alb. (g.)	Amount of normal gnpg's. serum (ccm)	Total quantity of fluid (ccm)	Result.
			A. Hypersensitive guineapigs.				
1	30	4	1:17	0,18	0,3	3,0	†
2	31	16	1:10	0,13	2,0	4,0	0
3	31	16	Res.	—	—	5,0	0
4	32	?	¹/₂₀	0,13	2,0	4,0	0
5	31	16	1:10	0,13	2,0	4,0	0
6	23	4	Lip.	0,18	0,35	3,0	†
7	18	16	Res.	—	—	5,0	0
8	14	20	0,09	0,09	2,0	4,0	0
9	32	12	1	0,1	2,0	4,0	0
10	39	20	0,046	0,09	2,0	4,0	0
11	15	?	Lip.	0,13	—	3,0	†
12	28	?	Lip.	0,13	—	6,0	†
13	13	16	0,05	0,09	2,0 (α)	4,0	†
14	14	16	¹/₂₀₀	0,13	2,0	4,0	†
			B. Normal guineapigs.				
15	—	48	Lip.	0,046	—	2,0	0
16	—	48	Lip.	0,13	—	6,0	sl S
17	—	16	Brain Res. from No. 5				0
18	—	16	Brain Res. from No. 4				0
19	—	24	0,06	0,06	1,0	2,5	S
20	—	24	0,06	0,06	1,0	2,5	S
21	—	48	0,07	0,08	1,2	3,0	S
22	—	48	0,07	0,08	1,2	3,0	S
23	—	24	0,08	0,1	6,0	7,5	0
24	—	24	0,06	0,09	5,5	6,5	0

(α) = inactivated serum.

Res. = Residue of Brain after decanting supernatant fluid of centrifuged mixture. Lip. = Suspension of lipoids extracted from brain.

The amounts of brain are given, either as 1 part of fresh brain to a given number of parts of mixture, or as a fraction of a brain, or as a given weight of dried brain substance.

guineapig's serum, 2) lipoid extract of brain, of hypersensitive guineapigs, egg albumin and normal guineapig's serum, 3) brain or lipoid and albumin only, and 4) the emulsion of the brain residue, after the supernatant fluid in the above mixtures had been removed. It will be seen that of the experiments in which sensitized guineapigs received injections of brain, egg albumin and normal serum (Nos 1, 2, 4, 5, 8, 9, 10 and 14), only two guineapigs (Nos 1 and 14) died. In all cases, the amount of egg albumin used was sufficient to kill. In Exp. I, only 0,3 ccm of normal serum was used, while in experiment 14, only a very small quantity of brain substance was used. With regard to the first point, when inactivated serum was substituted for fresh serum, the animal showed the usual signs of hypersensitization (No. 13), so that it would appear as if normal serum was necessary for the combination of the egg albumin and the receptor of the cell. The two experiments carried out with the residue of the brain, after the clear fluid had been removed, showed that the brain substance under these conditions either did not attract to itself the egg albumin or if it did so, was not in a condition to give up a toxic compound of egg albumin and receptor. It will further be noticed that when lipoid suspensions were substituted for brain substance, the prevention of symptoms was absent.

The negative results obtained in these experiments may be explained on the assumption that the egg albumin attaches itself to the sessile reaction product in the brain cells, and is therefore removed when the brain cells are sedimented by centrifugalization, i. e. that there is adsorption of the egg albumin, or on the assumption that the egg albumin is capable of attracting to itself the reaction product, in which case it would be incapable of combining with further sessile receptors in the brain cells of other sensitized guineapigs. The experiments on normal guineapigs tend rather to support the latter assumption, although the evidence which they yield is insufficient to form the basis for any far reaching theories. Further experiments will however doubtless clear up this point. From the second series, it will be seen that save when large amounts of normal serum were used, the mixture of egg albumin, brain and normal serum proved toxic for normal guineapigs. In each case, the fluid alone was injected, while in Nos 16 and 17, only the brain residue was injected with a negative result. One of these animals however was found to be hypersensitive at a later date, which clearly shows thas the brain cells had ad- or absorbed some egg albumin.

Before concluding I have the pleasant duty of recording my indebtedness to: the late Dr. Ludwig Mond, for his generosity and kindness in enabling me to carry out the research; to Dr. C. J. Martin, F. R. S., the Director of the Lister Institute of Preventive Medicine, for his valuable advice and supervision, and to my various colleagues at the Lister Institute for having been ready at all times to render help by advising and controlling.

Zusammenfassung.

Die Ergebnisse der vorliegenden Arbeit lassen sich etwa wie folgt zusammenfassen:

Das Ueberempfindlichkeitsphänomen kann leicht mit reinem Eiereiweiß hervorgerufen werden. Das Studium desselben wird dadurch begünstigt, daß man eine reine chemisch scharf abgegrenzte Substanz dazu verwendet. Im Vergleich mit Serum hat reines Eiereiweiß den großen Vorzug, daß die Reaktionen bezw. die krankhaften Erscheinungen durch eine einheitliche, statt eine komplizierte Substanz bedingt werden.

Die erste Dosis des Eiereiweiß darf beliebig groß sein, jedoch empfiehlt es sich, mit etwa 4—8 cg zu arbeiten. Die zweite Dosis kann gleichfalls klein oder groß gewählt werden, nur sind die Ergebnisse weniger konstant, wenn weniger als 6 cg eingespritzt werden.

Eine sogenannte passive Uebertragung der Ueberempfindlichkeit kann man auch mit Eiereiweiß bewirken. Das Serum überempfindlicher Meerschweinchen eignet sich allerdings nicht sehr gut zu diesem Zwecke, während das von vorbehandelten Kaninchen imstande ist, stets Ueberempfindlichkeit in normalen Meerschweinchen hervorzurufen.

Ein Serum kann auf seine Ueberempfindlichkeit übertragende Fähigkeit ungefähr geschätzt werden, indem die

minimale Dosis, die noch aktiv ist, festgestellt wird. Diese qualitative Beurteilung ist aber keine präzise.

Die von Victor Vaughan herstammende Behauptung, daß Eiklar des Hühnereies durch Behandlung mit alkoholischen Lösungen von Natronlauge in zwei Teile gespaltet werden kann, wovon dem einen die Fähigkeit innewohnt, Tiere überempfindlich zu machen, aber keine toxischen Symptome hervorzurufen, während der zweite letztere Eigenschaft nicht besitzt, dagegen die Erscheinungen der Ueberempfindlichkeit auslösen kann, gilt für das reine Eiereiweiß nicht. Die nach den Vorschriften Victor Vaughans sorgfältig vorbereiteten Spaltungsprodukte haben ihre anaphylaktisierenden, sowie ihre vergiftenden Eigenschaften fast gänzlich verloren.

Aus diesen sowie aus anderen Versuchen geht deutlich hervor, daß sämtliche Prozesse, die das Proteinmolekul zu spalten befähigt sind, die Eigenschaften des Proteins, Tiere überempfindlich zu machen und überempfindliche Tiere zu töten, im Verhältnis zu dem Grade der Spaltung einbüßen.

Das Reaktionsprodukt des Eiereiweiß besitzt den Charakter eines Ambozeptors. In vitro gewinnt eine Mischung von Eiereiweiß, Serum eines vorbehandelten Tieres und normalem Serum, toxische Eigenschaften, die Eiereiweiß allein nicht besitzt.

Es liegen Versuche vor, die zeigen, daß die Fähigkeiten eines Serums, Ueberempfindlichkeit hervorzurufen und Komplement abzulenken, ungefähr parallel laufen. Dagegen ist hervorzuheben, daß, obgleich einige Kaninchenseren imstande waren, in kleinen Dosen normale Tiere überempfindlich gegenüber Eiereiweiß zu machen, keins einen ausgesprochenen Präzipitingehalt darbot. Es lassen sich überhaupt mit reinem Eiereiweiß nur schwer Spuren von Präzipitin hervorrufen, und zwar nur dann, wenn alkalisch reagierende Lösungen verwendet werden. Im Meerschweinchenserum ist bisher keine Trübung, geschweige denn Fällung, beobachtet worden, aber dennoch wurden zeitweise Eiereiweißantikörper darin gefunden. Die Vermutung Friedbergers, daß nur ein Antieiweißkörper existiert, ist für Eiereiweiß noch nicht bewiesen.

Eine längere Serie von Versuchen ist dazu verwendet worden, um das Verhalten der Tiere nach überstandenem

Shock zu studieren. Daraus ergibt sich, daß das Ueberstehen deutlicher Symptome das weitere Bestehen der Ueberempfindlichkeit nicht zu stören braucht. Unter gewissen Bedingungen aber wird der tierische Organismus, vermutlich durch die Einwirkung von einem Ambozeptor-Eiweiß-Antikörper, außer stand gesetzt, auf weitere Einführung von Eiweiß zu reagieren.

Was das weitere Verhalten der Tiere nach der dritten Einspritzung anbetrifft, so ergibt sich aus den Versuchen, daß entweder eine wahre Immunität sich entwickelt, oder daß das refraktäre Verhalten eine Zeitlang anhält und wieder in Ueberempfindlichkeit übergeht. Eine gesetzmäßige Abwechselung ist nicht beobachtet worden, und die Lehre der Antianaphylaxie muß deshalb für Eiereiweiß in etwas modifiziert werden.

Es sind schließlich Versuche erwähnt worden, die die Annahme zu berechtigen scheinen, daß im Gehirn vorbehandelter Meerschweinchen sessile Rezeptoren existieren, denen dieselben Eigenschaften, wie den im Serum vorbehandelter Kaninchen und Meerschweinchen existierenden freien Ambozeptoren, innewohnen. Es sind dies aber nur vorläufige Versuche, die noch einer weiteren Bearbeitung harren.

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The Ætiology and Epidemiology of Paratyphoid Fever and "Food-poisoning."

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INTRODUCTION.

DURING the last few years a considerable advance has been made in our knowledge of the distribution of the bacilli associated with paratyphoid fever and with outbreaks of "food-poisoning." It seemed to me, therefore, that some account of the current views regarding the relation of these organisms to disease in man might be of interest to the members of this Section; these views will be discussed in the light of the recent improvements which have been made in the differentiation of the organisms concerned in these infections. I propose to confine myself as far as possible to the epidemiological aspect of the subject; but a preliminary reference to the bacteriology of these bacilli is desirable, since they are closely related to one another and their identification requires care.

BACTERIOLOGICAL DIFFERENTIATION.

There are four organisms which have often been found at one time or another in association either with cases of paratyphoid fever or with outbreaks of "food-poisoning" (in the clinical sense of the term). They are *Bacillus enteritidis* (Gaertner), *Bacillus suipestifer* (or *Aertryck*), *Bacillus paratyphosus* A and *Bacillus paratyphosus* B. I propose to deal with these bacilli only and to leave out of consideration *Bacillus botulinus* and other organisms.

The means by which these four bacilli are differentiated and identified are shown in the accompanying scheme :—

Description of organism	Cultural characters, including sugar tests	Agglutination test	Absorption test
(1) *Bacillus suipestifer* (or *Aertryck*)	Identical	Usually indistinguishable	Differentiated by this test
(2) *Bacillus paratyphosus* B ...			
(3) *Bacillus enteritidis* (Gaertner)		Stands alone	—
(4) *Bacillus paratyphosus* A ...	Differs from the others	Stands alone	—

Bacillus paratyphosus A can be readily identified by cultural methods and by agglutination tests with a suitable serum. The other three organisms are identical in their cultural characters, but *Bacillus enteritidis* (Gaertner) can be differentiated from *Bacillus suipestifer* and *paratyphosus* B by agglutination tests. Finally, *Bacillus suipestifer* and *Bacillus paratyphosus* B, which are usually—though not invariably—indistinguishable by the agglutination method, can be differentiated from one another by means of the absorption method introduced by Castellani.

It seems clear that all these bacilli can be differentiated and identified, if suitable methods are employed. Many German writers, however, do not use the absorption method for this group of bacilli ; as a result, they draw no distinction between *Bacillus paratyphosus* B and *Bacillus suipestifer*, but include these two bacilli under the term "paratyphoid" bacilli.

The fact that English writers regard *Bacillus suipestifer* and *Bacillus paratyphosus* B as distinct and separate bacilli, whereas some German workers regard them as identical, is of importance; it accounts to a large extent for the existing differences of opinion, both as to the distribution of these bacilli and as to their relation to disease in man.

HISTORICAL.

(1) As you are aware, *Bacillus enteritidis* was isolated by Gaertner in 1888, during an outbreak of "food-poisoning," both from the spleen

of a patient and from the meat which was regarded as the cause of the infection; this observation was the starting point of our definite bacteriological knowledge of "food-poisoning," although organisms of some kind had previously been obtained in similar outbreaks.

(2) The next step was taken by Durham and by De Nobele, who, almost at the same time in 1898, obtained from cases of "food-poisoning" and from the suspected food a bacillus which was identical in its cultural characters with *Bacillus enteritidis*, but could be distinguished from the latter by agglutination tests, then newly introduced. The bacillus was called *Bacillus Aertryck*, this being the name of the village in which occurred the outbreak recorded by De Nobele. Since that time it has been isolated in many outbreaks of "food-poisoning."

Further inquiry has also shown that *Bacillus Aertryck* is indistinguishable from *Bacillus suipestifer*—an organism which occurs in the alimentary canal of healthy pigs and in the tissues of pigs suffering from swine fever; and the two organisms are almost certainly identical.

(3) Finally, Schottmüller (1900-01) obtained two bacilli from the blood of patients whose clinical symptoms resembled those of enteric fever, and called them *Bacillus paratyphosus* A and B respectively. Similar bacilli had previously been isolated by Gwynn (1898) in a case of paratyphoid fever, by Achard and Bensaude (1896) from the urine of a case of clinical enteric fever, and by Widal (1897) from a thyroid abscess. During the last few years a large number of cases have been recorded.

EPIDEMIOLOGY.

(I) *Paratyphoid Fever.*

(1) *Bacillus paratyphosus* A.—With these introductory remarks, we may turn to the epidemiological significance of these organisms. *Bacillus paratyphosus* A may be very briefly considered. It has never been isolated in this country, and comparatively few cases have been recorded in Germany and America. In India, however, paratyphoid A fever appears to be not uncommon. Harvey has recently described ten cases in which this bacillus was isolated either from the blood or from the excreta; in their clinical aspect all the cases resembled enteric fever. Semple and Greig (at Kasauli) obtained *Bacillus paratyphosus* A from the blood or excreta of four cases, which also resembled enteric fever. One of these patients became a temporary carrier for some weeks after convalescence. In Sumatra, Baermann and Eckersdorff have recorded

eight cases of paratyphoid A fever, and a small epidemic of nineteen cases was observed in Paris. Up to the present no fatal cases have been recorded (apart from complication with other diseases), and the vast majority of the cases have resembled mild enteric fever, although *Bacillus paratyphosus* A has also been found in cases of acute enteritis. It has, however, been obtained on two occasions from the gall-bladder during operation for cholecystitis or gall-stones, and in one case from an abdominal abscess. With one exception, this bacillus has not been found in healthy men, in the lower animals, or in food or water. Both Morgan and Savage have obtained from the intestinal contents of healthy pigs an organism which is identical with *Bacillus paratyphosus* A in its cultural characters; but it is not agglutinated by paratyphoid A sera, and is probably a different organism. Since the distribution of *Bacillus paratyphosus* A is practically confined to patients with symptoms resembling those of enteric fever, or with disease of the gall-bladder, and since the presence of agglutinins for this organism in the serum of the patients points to an infection, it may fairly be regarded (in the absence of evidence to the contrary) as the causal organism of their illness. Very little is known concerning the existence of temporary or chronic carriers, but Harvey and Grattan have recently described a small outbreak (eight cases) of paratyphoid A fever which was apparently caused by a cook, who had recently had paratyphoid A fever and cholecystitis. The examination of his fæces and urine was negative, but the outbreak came to an end as soon as he was isolated.

(2) *Bacillus paratyphosus* B.—Schottmüller and other early writers believed that *Bacillus paratyphosus* B was the cause of the illness of those patients from whose blood it was isolated, and this view has been accepted by English writers (Boycott, Savage, Bainbridge) and by Seiffert and others. Recently, however, Conradi and Hübener have put forward the doctrine that the isolation of "paratyphoid" bacilli from the excreta (or even the blood) of a human being does not in the least indicate, in the absence of other evidence, that he has or has had paratyphoid fever. This conclusion is based upon their observations on the distribution of such bacilli in Nature. Thus Conradi found so-called "paratyphoid" bacilli in 29 out of 250 typhoid convalescents, in patients suffering from tuberculosis and other diseases, and in healthy individuals. Prigge and Sachs-Müke examined the fæces of 5,252 individuals, and found "paratyphoid" bacilli in 60 cases, apart from paratyphoid fever. Further, so-called "paratyphoid" bacilli have been found in sausages (Hübener, Rommeler), in milk, water, and in meat. Conradi also

noticed that food containing these bacilli could be consumed by human beings without ill-effects.

In criticism of the view of Conradi and Hübener it may be pointed out, in the first place, that the bacilli which they (and others) isolated were not submitted to absorption tests, and that it remains doubtful whether they were dealing with *Bacillus suipestifer* or *Bacillus paratyphosus* B. In point of fact, all such strains which we received from these writers have been proved by absorption tests to be *Bacillus suipestifer*. Secondly, most of those observations have been carried out in one district of Germany, and they are totally at variance with the results of other workers in Germany and in this country. In Berlin, Sobernheim examined 1,000 samples of fæces or urine from healthy men without once finding *Bacillus paratyphosus* B. Six hundred samples of human fæces were examined by Seiffert, with completely negative results. In this country Morgan investigated 303 samples of fæces from children suffering from summer diarrhœa; *Bacillus paratyphosus* B was not found. Savage failed to find *Bacillus paratyphosus* B in man, except in cases of paratyphoid fever. Dr. O'Brien and myself have recently investigated the fæces of a number of typhoid convalescents with completely negative results. The examination of the fæces of the lower animals, and of meat derived from such animals, has proved to be equally unsuccessful. Zwick and Weichel investigated 70 samples of apparently sound meat, and Müller examined 50 samples of meat from diseased animals (cows and calves); in no case did they obtain *Bacillus paratyphosus* B. The investigation of pigs' fæces by Morgan, by Savage, and by myself, also invariably yielded negative results.

In the hope of throwing further light on the distribution of *Bacillus paratyphosus* B, Dr. O'Brien and myself procured from various sources, mostly German, a number of strains of "paratyphoid" bacilli. Examination of these strains by cultural methods, by agglutination, and by absorption tests showed that they fell into two groups: one identical with standard strains of *Bacillus suipestifer*, the other identical with standard strains of *Bacillus paratyphosus* B. Further, all the strains of *Bacillus paratyphosus* B were derived from cases of paratyphoid fever or from chronic paratyphoid carriers, whereas all the strains derived from food or "food-poisoning" were identical with *Bacillus suipestifer*. Taking these facts into consideration, and bearing in mind that there is no conclusive evidence that the "paratyphoid-like" bacilli isolated by Conradi and others are really *Bacillus paratyphosus* B, it seems clear that the evidence strongly supports the view that the distribution of

Bacillus paratyphosus B is practically confined to man. It is probable, I think, that its normal habitat is the human alimentary canal (including the bile-passages), and that it is rarely (if ever) found in man, except in cases of paratyphoid fever and paratyphoid carriers. It may be admitted, however, that such carriers may cause infection not only directly but also by contaminating otherwise sound food, just as is the case with typhoid carriers.

This conclusion as to the distribution of *Bacillus paratyphosus* B is of epidemiological importance. In the first place, the evidence just detailed that this organism is found in cases of paratyphoid fever and under no other circumstances points to its being the actual cause of the illness of those patients in whom it is found. One ought to mention, however, that, as Schottmüller noticed in 1904, this bacillus is capable of giving rise not merely to paratyphoid fever but also occasionally to acute gastro-enteritis. An acute outbreak of this character has recently been recorded by Dr. Dudfield and myself. Secondly, the existence of chronic paratyphoid carriers (in man) brings us nearer to a rational explanation of the hitherto obscure epidemiology of paratyphoid fever. Lentz (1905-06) was apparently the first to call attention to these carriers; he recorded six cases, one of whom was under observation for at least a year. Similar cases have been observed by Hamilton (five cases), Prigge and Sachs-Müke (six cases), by Gaehtgens and by Meyer. Brückner recently examined the fæces and urine of 316 individuals who had previously had an attack of paratyphoid or enteric fever. He discovered nine typhoid carriers and three paratyphoid carriers; two of the latter were excreting paratyphoid bacilli three years after the original attack of paratyphoid fever. Prigge and Sachs-Müke found that all their cases give a positive serum reaction for *Bacillus paratyphosus* B. An analysis of the twenty-nine carriers recorded by these observers brings out two striking facts: twenty-six of the cases were women (the sex in one case is not stated), and seven of the cases were suffering from cholecystitis or gall-stones. Forster and Kayser, indeed, isolated *Bacillus paratyphosus* B from the gall-bladder of a woman; post mortem, gall-stones were found in her gall-bladder. It is obvious, therefore, that in their distribution as regards sex, in the frequent co-existence of biliary disorders, and in the presence of agglutinins in their serum, these carriers present a very close analogy with typhoid carriers.

The question of the capacity of paratyphoid carriers to cause infection has so far received very little attention. While investigating an epidemic of enteric fever, Wernicke (1907) found that 6 per cent. of the cases were paratyphoid fever; the cause of the epidemic was traced to a milkshop, in which resided (or worked) several typhoid carriers and one paratyphoid carrier. In the outbreak recorded by Dr. Dudfield and myself the evidence almost certainly excluded an ordinary food infection, and pointed to infection by a carrier; the blood of the individual whom we believed to be a carrier gave a positive serum reaction for *Bacillus paratyphosus* B long after the outbreak, although examination of the fæces (and urine) was negative. The recent outbreak of gastro-enteritis at Wrexham is also stated to have been caused by *Bacillus paratyphosus* B, and is attributed to infection by a human carrier.

Within the last year Sacquépée and Bellot have published an account of an epidemic of paratyphoid fever, and have given good grounds for attributing the source of infection to a carrier. In a corps of 250 soldiers in France, 19 cases of paratyphoid fever occurred in the course of eight days. The duration of the illness was from two to three weeks, and the clinical course was that of mild enteric fever; no deaths occurred. *Bacillus paratyphosus* B was recovered from the fæces or urine of 12 cases, and all the cases gave a positive serum reaction for this bacillus. An inquiry into the cause of the infection made it clear that the water supply could be excluded. It was found, however, that a man, who cooked the food for these soldiers, had suffered from paratyphoid fever a short time before the occurrence of the epidemic. His blood gave a positive serum reaction for *Bacillus paratyphosus* B, and this bacillus was obtained from his stools. Repeated examinations of his stools were made for some months, always with a positive result, and there can be no doubt that he was a paratyphoid carrier.

The foregoing evidence, incomplete though it is, as to the distribution of *Bacillus paratyphosus* B, the occurrence of persistent paratyphoid carriers, and the production of infection by such carriers, enables one to form a clear conception of the epidemiology of paratyphoid fever. The comparative infrequency of paratyphoid fever in this country (at least 3—6 per cent. of clinical enteric fever cases are really paratyphoid) and its favourable course make it difficult to obtain adequate material for investigation, but it is very desirable that, whenever such cases do occur, a careful search should be made for carriers.

(II) "*Food-poisoning.*"

(3) In dealing with the bacilli associated with "food-poisoning," one must bear in mind that the differentiation of *Bacillus enteritidis* (Gaertner) and *Bacillus suipestifer* by agglutination tests was not made until 1898. Except in those cases in which the strains were preserved until the introduction of the agglutination method the nature of the organisms isolated in the earlier outbreaks of "food-poisoning" remains doubtful. The available evidence concerning the association of *Bacillus suipestifer* with "food-poisoning" is further narrowed down by the omission of many writers to distinguish between *Bacillus suipestifer* and *Bacillus paratyphosus* B, and by their description of the bacilli found in such outbreaks as "paratyphoid" bacilli. On the present occasion I have taken into consideration only those outbreaks of "food-poisoning" in which convincing bacteriological evidence exists as to the nature of the organism which was present. In some outbreaks of "food-poisoning" *Bacillus suipestifer* was isolated both from the patients and from the food to which the illness was attributed; in others, it was obtained only from the food or from the patients. In most instances the bacillus was agglutinated by the serum (in high dilution) of the patients. Further, symptoms of illness were usually manifested only by those who had eaten certain articles of food; this food was almost invariably derived either from pigs or from cattle. In some cases the meat came from animals which had been ill, and were slaughtered for that reason; in other cases the animals were *stated* to be healthy.

Distribution of Bacillus suipestifer.—Recent observation has tended to raise doubts as to the validity of the foregoing evidence that *Bacillus suipestifer* causes "food-poisoning." In the first place, it has been stated that this organism has a wide distribution in Nature. Uhlenhuth obtained it from the fæces of 8 per cent. of healthy pigs, and similar figures were recorded by Seiffert. Savage, however, examined the excreta of a number of healthy cattle and horses, with completely negative results. I have recently failed to find *Bacillus suipestifer* in the fæces of fifty pigs. There is no doubt that *Bacillus suipestifer* occurs in apparently sound food, but the bacilli seem to be very scanty, and their presence is only demonstrable by special methods. Opinions differ as to their frequency in food, and the recent work of Sobernheim, Zwick and Weichel and Müller indicates that they are not often present. It is clear that, since it occurs in food, *Bacillus suipestifer* must from time to time be ingested by man; both Hübener and Conradi found that meat containing this bacillus could be eaten by human beings with impunity, and the bacilli could be obtained from the fæces of such people on the following day, but not subsequently. Prigge also noticed that those who ate such meat showed no sign of infection, and that their serum did not agglutinate the bacillus obtained from the meat. Although these bacilli are described as "paratyphoid" bacilli, my observations of strains derived from food leads me to believe that the writers were dealing with *Bacillus suipestifer*. The evidence for the distribution of *Bacillus suipestifer* in man is conflicting. Sobernheim and Seiffert failed to obtain it from the excreta of healthy men, whereas Conradi regards the presence of "paratyphoid" bacilli as of frequent occurrence. In this country it has very rarely been found in man apart from outbreaks of "food-poisoning," although Murray, Williams, and Rundle have recently isolated it in seven out of forty cases of summer diarrhœa. From our own observations, Dr. O'Brien and myself are disposed to believe that the usual habitat of *Bacillus suipestifer* is the alimentary canal of pigs and other lower animals, and food derived from such animals, although it is not very often present even in animals and food. The evidence as a whole suggests that *Bacillus suipestifer* has a regional distribution, occurring much less frequently in some districts and countries than in others. Whether outbreaks of "food-poisoning" occur more often in those districts in which this organism is most widely distributed is not known. But it seems probable that in this country and in some parts of Germany, *Bacillus suipestifer* is *not* widely distributed either in healthy animals or food or healthy men. There are other facts, however, which arouse scepticism as to its capacity to initiate an infection. The observations of Dorset and Bolton (confirmed by Uhlenhuth, Hübener and Xylander) have made it perfectly clear that swine fever is caused by a filter passer, and that *Bacillus suipestifer*, which was at one time regarded as the cause of the disease, is merely a secondary invader. Petrie and O'Brien showed that it played an equally minor part in an epizootic among guinea-pigs, which was probably caused by a filter passer. It is further stated that the consumption by man of the flesh of pigs dead of swine fever has rarely given rise to symptoms of "food-poisoning," although presumably *Bacillus suipestifer* was sometimes present in the meat.

Relation of Bacillus suipestifer to "Food-poisoning."—In forming an opinion as to the relation of *Bacillus suipestifer* to "food-poisoning," two groups of facts must be taken into consideration. On the one hand, this organism can frequently be isolated from the excreta of patients suffering from "food-poisoning," while it is very rarely found in healthy people, at least in England. The outbreak can often be traced to a given article of food, and the same bacillus can be found in the food. On the other hand, there is evidence that meat containing *Bacillus suipestifer* can sometimes be eaten without ill-effects, and that in other diseases it is merely a secondary invader and does not initiate the illness. It must be admitted, I think, that *Bacillus suipestifer* has some relation to "food-poisoning," and that it must be either a secondary invader or a causal organism of the disease. If *Bacillus suipestifer* is a secondary invader in "food-poisoning" in man, the primary cause of the disease may be either a filter passer or a ptomaine. There is no direct evidence for the presence of a filter passer; and, unlike filter-passer infections, "food-poisoning" is not contagious. Nor has the hypothesis that "food-poisoning" is caused by ptomaines received any support during the last few years. The evidence for this view is still slight and indefinite; and, so far as I know, no one has yet demonstrated the presence of ptomaines in food which had caused "food-poisoning." I am inclined, therefore, to think that *Bacillus suipestifer* probably actually causes "food-poisoning," although it is not yet possible to reach a final conclusion on the question. But it appears likely that it is always potentially dangerous to man, and that chance, or perhaps the number of bacilli ingested, determines whether food containing this organism does or does not set up disease. We know that *Bacillus typhosus* is occasionally taken in by the mouth and excreted without giving rise to clinical symptoms of disease. It has also been noticed that, when food containing *Bacillus suipestifer* was eaten without ill-effect, the number of bacilli in the food was extremely small. Even during outbreaks of "food-poisoning," individuals are met with who remain in good health although they have eaten the suspected food and are excreting *Bacillus suipestifer* or *Bacillus enteritidis* in their fæces. One may perhaps regard the occasional ingestion of this bacillus by man without ill-effect as showing that the individual was less susceptible rather than that the bacillus was non-pathogenic.

(4) *Bacillus enteritidis* (Gaertner).—The relation of *Bacillus enteritidis* to "food-poisoning" is not difficult to establish, since it can be identified with certainty, and there is abundant and reliable evidence as to its distribution. I have met with accounts of eleven outbreaks of "food-poisoning" in which this bacillus was obtained either from the patients or from the food to which the illness was attributed, or from both the food and the patients. The nature of the bacillus was ascer-

tained in all cases by cultural and agglutination tests. In one outbreak the cause of the illness was said to be brawn, in another pork sausages; the remainder were due to veal or beef, and in eight outbreaks the animal (whose flesh was eaten) was ill at the time when it was slaughtered. The fact that so many outbreaks were apparently caused by meat derived from diseased animals seems important, since their illness would tend to raise the virulence of *Bacillus enteritidis* and to favour its extension into the tissues of the animals. Apart from these outbreaks, *Bacillus enteritidis* has seldom been isolated from the organs of cattle which have died of disease or been killed in emergency. Müller, Edenhuizen, and Portet examined the organs of healthy or diseased cattle without finding *Bacillus enteritidis.* Nor was it obtained by Savage from the fæces of cattle, sheep, and pigs. Sobernheim examined the organs of a very large number of healthy animals, and twice isolated *Bacillus enteritidis.* This organism is of rare occurrence, not only in animals but also in man. It has been found only five times in human fæces—four times by Morgan in cases of summer diarrhœa, and once by Savage in a case of typhoid fever. Epizootics associated with the presence of *Bacillus enteritidis* have been observed by Boycott, Dunbar, Bainbridge, and others in rats and rabbits, and by McConkey in guinea-pigs; this bacillus was apparently the actual cause of all the epizootics.

During the last few years bacterial viruses have been extensively employed for the destruction of rats; many of these viruses are either pure cultures of *Bacillus enteritidis* or consist of foodstuff containing this bacillus. The administration and distribution of these viruses by unskilled persons must almost inevitably expose those who handle the virus to some risk of infection, and Handson and Williams have reported an outbreak of "food-poisoning" in which they consider that food had become infected by one of the viruses. No *direct* evidence of such infection was obtained, but the epidemic occurred among persons who took their meals in the room in which the virus had been placed.

Outbreaks of disease in man traceable to infection by a virus are, however, very infrequent. Many samples of these viruses are only slightly virulent even for rats, and the dose of virus which those who handle it are likely to ingest is probably small, particularly if the usual instructions are followed. Moreover, occasional individual instances of infection might easily escape notice and fail to be recorded. These reasons help to explain why *Bacillus enteritidis* has so seldom caused disease, in spite of its extensive employment as a virus.

There can be little doubt, from the evidence just detailed, that *Bacillus enteritidis* can cause "food-poisoning." Further information is needed, however, concerning its distribution in the lower animals and the possible existence of animal carriers.

It thus appears that *Bacillus enteritidis*, *Bacillus suipestifer*, and *Bacillus paratyphosus* B can all give rise to the clinical symptoms of "food-poisoning." In the case of *Bacillus paratyphosus* B the usual source of infection is probably food which has been contaminated by a human carrier; in the case of *Bacillus suipestifer* and *Bacillus enteritidis* (Gaertner) the source of infection is food derived from healthy or diseased animals, and containing these bacilli from the outset. The two latter organisms are probably responsible for the majority of food-poisoning outbreaks. In outbreaks associated with the presence of *Bacillus enteritidis* the incriminated food has usually been derived from diseased cattle; in outbreaks associated with *Bacillus suipestifer* the food has been derived from cattle or pigs. The possibility that such outbreaks may be caused by bacterial viruses must not be overlooked.

In conclusion, it is obvious that these facts have a practical value for those who are called upon to investigate outbreaks of "food-poisoning." It is of importance to ascertain what organism is present in such outbreaks, since that knowledge gives a clue to the most probable source of the infection. Depending upon the nature of the bacillus which is found, attention can be at once directed to the kind of food in which the bacillus usually occurs, or a search may be made for a human carrier.

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Nº 4

AN OUTBREAK OF ACUTE GASTRO-ENTERITIS CAUSED BY *B. PARATYPHOSUS* (B.).

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History of the Outbreak.

On June 14th, 1910, the attention of one of us was directed by the House Physician of the Hampstead General Hospital, to an outbreak in Paddington of what was thought to be "food-poisoning." Two of the patients were at the time under his care.

The history of the outbreak was briefly as follows: On the night of the 10th—11th June, eight persons out of twenty-nine living in a high-class boarding-house were taken ill, five of the cases being amongst the servants, seven in number, and three among the guests, twenty in number. The two proprietors of the house were not affected. All the patients were females and with one exception (aetat. 15) adults, some of mature age.

The symptoms were practically the same in all the cases, but one patient was much less affected than the rest. She had, by coincidence, taken a purgative on the night of the 9th and her escape from an attack of a severity equal to that of the others may be attributed to that cause. All had severe abdominal pain, followed by vomiting and persistent and violent diarrhoea, which could not be controlled by the usual remedies. Fever supervened, the temperature rising as high as 103° F. in some cases, and lasting for various periods up to a week. There were no indications of any pulmonary lesions, or of any splenic enlargement, nor was there any delirium. Some of the more elderly patients had attacks of grave syncope, and, with the exception of the case already mentioned, convalescence was slow. All the patients recovered.

The first inquiries were naturally directed to find out what food had been the cause of the outbreak. The time of the onset of the outbreak and the fact that none of the male guests had been attacked suggested that the infected food—if there had been any—had been eaten at the midday meal of the 10th at which only one male guest had been present. Careful inquiries, however, failed to elicit any evidence of any item of the menu of that meal having been taken by the patients and passed by those who escaped. Still, in view of the well-known capriciousness manifested in the attack and escape of persons partaking of infected food, it was thought advisable to pursue inquiries with relation to each article of food provided at the meals of the 9th and 10th. The results of the inquiries were entirely negative and while they were in progress the bacteriological examinations, which are described below, led us to the conclusion that food infection had not been operative.

Samples of the faeces from two patients were received on the 14th and forwarded to the Lister Institute, the patients then having been three days ill. It was not known at the time that one of the patients had been treated with saline irrigations of the rectum, but that information subsequently explained the fact of her faeces yielding practically no growth. The specimens when received were quite fluid and of a brilliant green colour. A third sample was received on the 17th from a patient then ill six days, who had had 14 or 15 motions on that day. This patient (Miss L.) was upwards of 70 years old, and hers was one of the severest cases of the outbreak. She had several attacks of heart failure, and her case gave rise, at one time, to much anxiety.

Specimens of blood were obtained from six of the patients and submitted to agglutination tests at the Lister Institute.

In the course of the investigation, information was received of similar cases in Willesden, Hampstead and Lambeth which were thought to have been connected with the Paddington outbreak. Details of those cases were obtained from the Medical Officers of Health of the districts named and such information is summarised in the following paragraphs:—

(1) A group of cases occurred in the family (named H.) of the temporary cook at the boarding-house, four persons being taken ill during the morning of the 14th. All had abdominal pain and diarrhoea, but

vomiting was not present in all cases. There was a history of dried haddock being eaten at supper on the night of the 13th but, in the light of other evidence, the causal relationship between the fish and the illness appears to be doubtful. Specimens of the blood from two of the patients (Mr and Miss H.) were submitted to agglutination tests.

(2) A laundry proprietor, named T., also residing in Willesden, who called at the boarding-house on the 10th, had diarrhoea on that and the following two days, which he attributed to eating a veal and ham pie on the 10th. There was no history of any illness among the other members of T.'s family.

(3) A further case in the same district was reported in the person of a woman who was at the time of the outbreak kitchen-maid at the boarding-house, but, as the woman was said to have taken a purgative just before her attack of diarrhoea, the case was not (unfortunately) followed up.

(4) The case in Hampstead was in the person of a married sister (Mrs R.) of the temporary cook at the boarding-house, with whom the cook lived. Mrs R. visited her mother Mrs H. in Willesden on the 13th and the same evening had severe abdominal pain, followed in the morning by vomiting and diarrhoea. Mrs R. did not share the haddock supper to which the H.'s attributed their illness, but she had a piece of cold fried fish brought from the boarding-house on the previous Sunday (12th). An unavailing attempt was made to secure a specimen of Mrs R.'s blood.

(5) The Lambeth case was that of Mrs D. who had been married only a short time, leaving a situation in the boarding-house for that purpose. She visited the house on Monday June 27th to see one of the servants, had "tea" in a bedroom in which one of the original patients had been nursed and had an attack on the following day, which is best described, in the absence of medical information, in her own words. She wrote to her friend: "I have been so ill since I saw you on Monday. All day Tuesday and Wednesday I felt as though I was going to die. I was sick and all the other (*sic*) all day and night, doubled up with pain and all that came up was like thick green paint." Such account suggests that Mrs D. had a pretty severe attack of diarrhoea and vomiting, much the same as the inmates of the boarding-house. The Medical Officer of Health of Lambeth reported that Mrs D.'s medical attendant described the case as one of ptomaine poisoning. The "tea" referred to is said to have consisted of tea and bread and butter—no potted meats. A specimen of Mrs D.'s blood was refused.

Bacteriological Examination.

(*a*) Three samples of faeces were received during the outbreak, two on June 15th from Miss G. and Miss W. and one on June 17th from Miss L. When examined by the methods usually employed for such cases, the faeces of Miss W. yielded no growth, but the faeces of Miss G. and Miss L. yielded cultures which were identical in their cultural characters with *B. paratyphosus* (B.). The nature of these cultures was determined by observing their agglutination and absorption reactions with various sera. The results which are given in Tables I and II show that both the strain "G" from Miss G., and the strain "L" from Miss L. were indistinguishable from standard strains of *B. paratyphosus* (B.).

These results show that the bacilli isolated from the patients' faeces were *B. paratyphosus* (B.). Strain G was found to be highly virulent

TABLE I. *Agglutination tests.*

The Macroscopic method was used, and the agglutination limits were observed after incubation for 2 hours at 37° C. Controls were made in every case.

	Serum		
Organism	*B. paratyphosus* (B.)	*B. suipestifer*	*B. Gaertner*
B. paratyphosus (B.), McWeeney	20,000	5,000	<100
B. suipestifer (Laboratory strain, Lister Institute)	5,000	10,000	<100
Strain G 1	20,000	5,000	<100
Strain G 2	20,000	5,000	<100
Strain L	20,000	5,000	<100
B. Gaertner (original strain of v. Ermengen)	<100	<100	5,000

TABLE II. *Absorption tests.*

Macroscopic method. Agglutination limits after absorption, observed after incubation for 2 hours at 37° C. Controls made in every case.

	Organisms			
Serum	*B. suipestifer*	*B. paratyphosus* (B.)	Strain G	Strain L
I. *B. suipestifer.* Original titre	5,000	5,000	5,000	5,000
B. suipestifer. Absorbed with *B. paratyphosus* (B.)	2,000	<100	<100	<100
II. *B. paratyphosus* (B.). Original titre	5,000	20,000	20,000	20,000
B. paratyphosus (B.). Absorbed with *B. suipestifer*	<200	10,000	10,000	—

for guinea-pigs, since 1/1000 c.c. of a 24-hour broth culture, injected intraperitoneally, killed in three days.

The faeces of patients G. and L. were filtered through a Berkefeld filter, and a dose of 2 c.c. of the filtrate was injected into a guinea-pig without producing any obvious effect.

(*b*) Blood was obtained from six of the patients at intervals of 10—20 days after the onset of the symptoms and agglutination tests were carried out for strain G., *B. suipestifer* and *B. paratyphosus* (B.). It will be seen from Table III that, with one exception, all the patients' blood agglutinated these three organisms and as a rule to the same extent.

The serum of the patients was pooled, and the combined serum was absorbed with *B. suipestifer* and *B. paratyphosus* (B.) and its agglutination limits after absorption were ascertained (Table IV).

TABLE III. *Agglutination tests carried out with the patients' sera.*

Wright's method; controls were made in every case.

		Organism		
	Patients' serum	*B. paratyphosus* (B.)	*B. suipestifer*	Strain G
(1)	Miss G. 1st sample	1—200	1—200	1—200
(2)	,, 2nd ,,	1—400	—	1—400
(3)	B. B.	1—400	1—400	1—400
(4)	Miss W.	1—200	1—200	1—200
(5)	Mr H.*	1—400	1—400	1—400
(6)	Miss H.*	1—400	1—400	1—400
(7)	Mrs W.	1—100	1—100	1—100
(8)	Mr S.	—	—	<1—40
(9)	Miss L.	<1—200	<1—200	<1—200

* These patients resided at Willesden, not at the boarding-house.

TABLE IV. *Pooled serum. Absorption test.*

Wright's method. Agglutination limit after absorption, observed after incubation for 2 hours at 37° C. Controls made in every case.

		Organisms		
	Serum	*B. suipestifer*	*B. paratyphosus* (B.)	Strain G
Pooled serum.				
(1)	Original titre	100	100	100
(2)	After absorption with *B. suipestifer*	<20	80	80
(3)	After absorption with *B. paratyphosus* (B.)	<40	<40	<40

CONCLUSIONS.

It thus appears that, while the bacteriological evidence pointed to only one organism, viz. *B. paratyphosus* (B.), as the cause of the infection in all cases, no one article of food had been eaten by all the patients and the only link between the primary outbreak in Paddington and the secondary cases in Willesden, Hampstead, and Lambeth, was furnished by the exchange of visits between the different houses. A consideration of these facts made it clear that the outbreak was almost certainly not caused by food, and an attempt was made to find a paratyphoid-carrier. The existence of paratyphoid (B.) carriers is now well known (Lentz [1905], Prigge and Sachs-Muke [1909]), and there are grounds for supposing that this organism has its habitat in the human alimentary canal. It was impossible, however (as is often the case in such outbreaks), to obtain the necessary material (blood and faeces) from those members of the boarding-house, who had not been ill, and no actual carrier was found.

For a time suspicion fell upon a servant, who had entered the boarding-house only a few days before the outbreak took place, and who had remained at work during the outbreak (although she had a mild attack of diarrhoea); but the examination of her urine and faeces always yielded a negative result as regards *B. paratyphosus* (B.) although her blood still agglutinated this organism up to 1—100 four months after the outbreak.

Nevertheless, the outbreak is of interest for two reasons. In the first place, it is clear that, as Schottmüller (1904) pointed out, *B. paratyphosus* (B.) is capable of giving rise not only to paratyphoid fever, but also to acute gastro-enteritis simulating "food-poisoning," a fact not hitherto observed in this country. Secondly, the distribution and dates of onset of the illness of the various cases were unlike those of ordinary "food-poisoning," and pointed to a human source of infection.

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N°5

ON THE PARATYPHOID GROUP OF BACILLI.

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(From The Lister Institute of Preventive Medicine.)

CONTENTS.

PAGE
Introduction 68
Distribution of "paratyphoid" bacilli in man 69
,, ,, ,, animals and food . 70
The scope of the present enquiry 71
Description of the organisms examined 72
Methods employed 73
Results
(*a*) Examination of typhoid convalescents . . . 74
(*b*) Examination of strains of the paratyphoid group . 76
(1) Cultural characters 76
(2) Agglutination reactions 76
(3) Absorption tests 78
Discussion of results 83
Summary and conclusions 86

Introduction.

THERE exists at the present time considerable difference of opinion both as to the distribution in nature of *B. paratyphosus* (B) and as to its relation to disease in man. The name was given by Schottmüller (1900 and 1901) to a bacillus, which he isolated from patients whose clinical symptoms were those of enteric fever, and which he regarded as the causal organism of their illness. Further enquiry soon showed that this bacillus was closely related to *B. suipestifer* (or *aertryck*) which occurs in the intestine of healthy pigs and has been found on several occasions in association with outbreaks of food-poisoning in man. Almost all writers in this country, however, regard them as distinct organisms which can be differentiated from one another sometimes by their agglutination reactions and always, as both Boycott (1906) and Bainbridge (1909) have shown, by the absorption method; and most workers appear to have experienced no difficulty in identifying the strains which they isolated as *B. suipestifer* or *B. paratyphosus* (B) respectively. The majority of English writers therefore limit the term *B. paratyphosus* (B) to those bacilli which correspond in all respects, including their reaction to absorption tests, to original strains of the bacillus isolated by Schottmüller.

On the contrary Kutscher and Meinecke (1906), Bock (1906) and others regard *B. suipestifer* and *B. paratyphosus* (B) as indistinguishable from one another, and as being in all probability identical organisms; with the exception of a few observations by Bock, who was able to differentiate *B. paratyphosus* (B) from *B. suipestifer*, the absorption method was apparently not employed by these observers. This conclusion has been generally accepted by German writers, who almost without exception describe as *B. paratyphosus* (B) all bacilli whatever their source may be, which correspond in their cultural and agglutination reactions to the "paratyphoid" group; and they draw no distinction between *B. suipestifer* and *B. paratyphosus* (B), although they retain the term *B. suipestifer* for the organisms of this group which are derived from pigs.

Distribution in man.

Since some writers draw a distinction between *B. suipestifer* and *B. paratyphosus* (B), whereas others do not, and since the term *B. paratyphosus* (B) is not used in the same sense by different writers, it is hardly to be expected that they should agree as to the distribution and pathogenic significance of "paratyphoid" bacilli. The evidence regarding the distribution of these bacilli in man is, indeed, remarkably conflicting. On the one hand a very complete investigation of the faeces of 303 cases of summer diarrhoea by Morgan (1905–1907) yielded only two strains which gave the cultural and agglutination reactions characteristic of *B. suipestifer*. Savage (1909) examined sixty samples of faeces and urine from typhoid patients and from healthy individuals, but failed to find paratyphoid (B) bacilli in man except in cases of paratyphoid fever; Williams, Rundle and Murray (1910) examined the stools of 100 healthy children with completely negative results. Nor was *B. paratyphosus* (B) found either by Seiffert (1909) who examined the faeces of 600 healthy men, or by Sobernheim (1910) who investigated 1,000 samples of urine and faeces from healthy men. The conclusion, to which these observations point, is that the paratyphoid (B) bacillus is very rarely found in the human intestine or in urine apart from cases of paratyphoid fever.

On the other hand, many German workers have brought forward evidence that "paratyphoid" bacilli (in the wider sense of the term) can be obtained from the faeces, the urine and even the blood of healthy individuals. Thus, Conradi (1909) isolated "paratyphoid" bacilli from the faeces of twenty-nine out of 250 typhoid convalescents, from several typhoid patients and "contact" cases, from patients suffering from other diseases, and from healthy individuals. In the course of some years, Prigge and Sachs-Müke (1909) examined the faeces or urine (or both) of 5,252 people, and found "paratyphoid" bacilli in seventy cases, apart from cases of paratyphoid fever. Similar observations, though of a less striking character, have been published by Gaethgens (1907) and others.

Distribution in animals and food.

The distribution of the "paratyphoid" group of bacilli in food and in the lower animals has received hardly any attention in this country, and no systematic search for these organisms appears to have been carried out[1].

Recent observations by Petrie and O'Brien (1910), however, point to the frequent occurrence of *B. suipestifer* in healthy guinea-pigs, and O'Brien (1910) has met with several guinea-pigs which were persistent carriers of this bacillus. In the course of the bacteriological examination of 850 glucose-fermenting bacilli obtained from many samples of milk (number not stated), Orr (1908) found eight strains which gave the cultural reactions characteristic of the "paratyphoid" group, but were not subjected to agglutination tests. Observations on the presence of these organisms in meat seem to be entirely lacking.

In Germany, bacilli of the "paratyphoid" group have been found in milk by Fischer (1903), in water by Conradi (1904), Priefer and

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[1] One of us (F. A. B.) has recently examined the intestinal contents of 50 healthy pigs; no bacilli of this group were found.

Keyser (1902), in sausages by Hübener (1908) and by Rommeler (1909), and in goose (Spickgänse) by Sobernheim (1910). They have also been obtained from the intestinal contents of healthy pigs by Uhlenhuth (1908) (8 °/₀) and by Seiffert (1909) (3·5 °/₀).

On the ground of this evidence and of their observation that the consumption by man of food containing these bacilli led to no ill effects, Hübener and Conradi consider not only that "paratyphoid" bacilli have a wide distribution in nature, but also that they are usually harmless for man, and that the isolation of these bacilli from the excreta of a human being is no indication that he has or has had a paratyphoid infection, and is in fact of no special significance.

It seemed to us possible that an enquiry into the distribution of *B. suipestifer* on the one hand and of *B. paratyphosus* (B) on the other hand might furnish some evidence as to their pathogenic significance, and might also assist in explaining the opposing views now held by various workers. The present investigation was carried out from this point of view.

The scope of the present enquiry.

Although the cultural characters of *B. enteritidis* (Gaertner) are identical with those of *B. suipestifer*, both *B. Gaertner* and *B. paratyphosus* (A) are clearly marked off from *B. suipestifer* and *B. paratyphosus* (B) by their agglutination reactions. They have, therefore, been left out of consideration in this paper, and the term "paratyphoid group of bacilli," as here used, includes only *B. suipestifer* and *B. paratyphosus* (B).

The investigation of the "paratyphoid" group has been carried out along two lines. In the first place, the faeces and urine of a large number of typhoid convalescents were examined in order to obtain some evidence as to the prevalence of bacilli of the "paratyphoid" group in the human alimentary canal, apart from cases of paratyphoid fever.

Secondly, a considerable number of strains of the "paratyphoid" group of bacilli was obtained from various sources. They were compared with standard, recognised strains of *B. suipestifer* and *B. paratyphosus* (B) as regards their cultural characters and their agglutination and absorption reactions. Attention was particularly directed to their absorption reactions, since as previously mentioned it has been shown by Boycott (1906) and also by Bainbridge (1909) that *B. suipestifer* and *B. paratyphosus* (B) can be readily differentiated from one another by means of the absorption method of Castellani (1902), and that the use of this method is essential for the complete identification of any given strain belonging to the "paratyphoid" group.

Description of the organisms examined.

The following strains have been examined:—

A. *Standard strains.*

	Description	Source
(*a*)	*B. paratyphosus* (B)	Schottmüller; original strain 1901.
(*b*)	,,	Professor McWeeney: isolated from a fatal case of paratyphoid fever.
(*c*)	*B. suipestifer*	Laboratory strain (Kral).
(*d*)	,,	Isolated from a healthy pig: sent by Professor Uhlenhuth.
(*e*)	,,	Sent by Professor Wassermann.

These strains had been repeatedly tested by the absorption method and always yielded constant results.

B. *Fresh strains.*

	Description	Source
(1)	Eisenmeyer	Paratyphoid fever case.
(2)	Bieber	Paratyphoid carrier (Dauerträger).
(3)	Kunz	,, ,, ,,
(4)	Vogt	,, ,, ,,
(5)	Schnorr	,, ,, ,,
(6)	Kant	Healthy man.
(7)	Ma	,, ,,
(8)	Düsseldorf	"Sick" man.
(9)	Heydr. Ali	,, ,,
(10)	Ba	Food.
(11)	Liver	Liver cheese (Leberkäse).
(12)	Sausage	Blood sausage (Blutwurst).
(13)	Murrow	Food-poisoning (patient).
(14)	Savage, *c*	Paratyphoid fever case.
(15)	,, *w*	,, ,, ,,
(16)	Lyons	Food-poisoning (patient).
(17)	Winfield	Paratyphoid fever case.
(18)	Gill	Gastro-enteritis (patient).
(19)	Zender	Healthy man.
(20)	Lieblang	Paratyphoid fever case.
(21)	Lister	Water.
(22)	Bacillus F	Summer diarrhoea.
(23)	Victoria	Fatal Ac. gastro-enteritis (?food).
(24)	Garton	,, ,, ,,

Methods employed.

The mode of carrying out the cultural, agglutination and absorption tests has been described in detail in a former paper (Bainbridge, 1909) and reference need be made only to two points. In the first place, some difficulty was experienced in obtaining highly agglutinating sera by the injection of *killed* cultures into rabbits, and the sera agglutinated equally both *B. suipestifer* and *B. paratyphosus* (B). Sera, obtained from rabbits injected with *living* cultures, had a very high agglutination titre for the homologous organism, and a distinctly lower titre for heterologous strains.

Secondly, attention may be called to Dreyer's observation that the absorption of agglutinins consists partly in a specific combination of agglutinin and bacteria, and partly in a non-specific or less specific physical absorption of agglutinin. It is possible by the addition of very large amounts of a heterologous bacillus to remove some of the homologous agglutinin from a serum, but we have not succeeded in removing all the homologous agglutinin in this way. The difference between the amount of heterologous bacilli which must be added to a serum to absorb only the heterologous agglutinin, leaving the homologous agglutinin intact, and that necessary to absorb much of the homologous agglutinin, is so large that errors cannot occur if reasonable care is taken (as may be seen in Tables I and II). As a rule, one or two trials suffice to determine the amount of heterologous bacilli, which must be added to a serum in order to remove the heterologous agglutinins —and so get the specific effect—without appreciably affecting the homologous agglutinin; when this amount has been once ascertained, the result can be reproduced without difficulty on subsequent occasions. It is also safer and preferable to measure the agglutination limit of the serum after absorption rather than merely to observe the agglutination at one or two dilutions, and it is important in every experiment to test the absorbed serum against standard strains as well as against the organism under investigation. The absorption method is also much more easily carried out, and the results obtained are much sharper, when sera are used which have a high agglutination titre (10,000 or more).

The examination of the faeces and urine of the typhoid convalescents was carried out in the following manner: the material was plated out on lactose-neutral-red-bile-salt agar and a number of white colonies were picked off into mannite broth by Dr Ledingham, who kindly handed on to us all cultures which formed acid and gas in this medium. The cultures were replated on lactose-saccharose-bile-salt-neutral-red agar, and the colourless colonies were picked off into dulcite broth. The cultures, which fermented dulcite, were then tested by all the ordinary cultural tests used for identification of bacilli of the "paratyphoid" group.

TABLE I. *The effect of progressive absorption of a serum with heterologous bacilli.*

A Paratyphoid (B) serum was absorbed with *B. suipestifer*, the bacilli being added from the agar slopes.

	Agglutination limits after absorption		
Paratyphoid (B) serum	*B. paratyphosus* (B)	*B. suipestifer*	*B. aertryck*
Original titre	4,000	1,000	1,000
1st absorption with 3 slopes ...	4,000	<200	<200
2nd ,, ,, 3 further slopes	2,000	<100	<100
3rd ,, ,, 3 ,, ,,	1,000	—	—

TABLE II. *The effect of absorbing a serum with varying amounts of heterologous bacilli.*

The volume of serum used was constant, namely 2 c.c. of 1—10 dilution; the amount of bacilli added varied from 2—8 agar slopes of approximately uniform size and density of growth.

A *B. suipestifer* serum was absorbed with *B. paratyphosus* (B).

	Agglutination limits after absorption	
Serum	*B. suipestifer*	*B. paratyphosus* (B)
B. suipestifer serum. Original titre	20,000	5,000
,, absorbed with 2 slopes	20,000	<100
,, ,, 4 ,,	20,000	<100
,, ,, 8 ,,	20,000	—

Results.

(*a*) *Examination of typhoid convalescents.*

It is now generally admitted that *B. suipestifer* and *B. paratyphosus* (B) are indistinguishable by their cultural reactions. Briefly these reactions are as follows:

(1) The production of acid and gas in broth containing glucose, mannite, dulcite, laevulose, galactose, arabinose, maltose and sorbite.

(2) No apparent change in broth containing lactose, saccharose, salicin, raffinose and inulin.

(3) Litmus milk shows an initial transient acidity not often lasting more than 24 hours; in 3—5 days the milk is definitely alkaline and at the end of a week intensely alkaline; no clot is formed, and there is no obvious sign of digestion.

(4) No indol is formed.

(5) Neutral red broth is usually reduced in 48 hours, but this test is less reliable than the others.

When subjected to these tests, not one of the 341 colonies which we obtained from the faeces or urine of approximately 300 typhoid convalescents, corresponded in its cultural characters to *B. paratyphosus* (B). A large proportion of them fermented saccharose, and the remainder produced indol and fermented salicin. Several of the strains, which most nearly resembled *B. paratyphosus* (B) in their cultural characters, were tested with Gaertner and paratyphoid (B) sera, but none of them was agglutinated in dilutions higher than 1—100 (Table III). Similar results were obtained by Savage (1909). We have in fact never met with any organism which was agglutinated beyond the above limit by a paratyphoid (B) (or Gaertner) serum, unless its cultural and fermentation characters corresponded in all respects with those bacilli.

TABLE III. *Agglutination reactions of strains isolated from typhoid convalescents. These strains resembled but were not completely identical with B. paratyphosus (B) in their cultural reactions.*

Strain	Paratyphoid serum (titre 20,000)	Gaertner serum (titre 5,000)
1. Lythe	<100	<100
2. Crowdy	<100	<100
3. Newman	<100	<100
4. B. B. 3	<100	—
5. K. 4	<100	—
6. Roberts, 2	<100	—
7. Robinson	<100	—
8. B. 270	<100	<100

These results entirely confirm those of Morgan (1907), Seiffert (1909), Savage (1909), Williams, Rundle and Murray (1910), as opposed to those of Conradi (1909), Gaethgens (1907) and Prigge and Sachs-Müke (1909). There is no reason to suppose that the differences in the technique adopted by different writers for the *isolation* of this group of bacilli account for these conflicting results. The differences consist almost entirely in the medium used for plating out the material under investigation; some workers used lactose-bile-salt-neutral-red agar, others use malachite green and Conradi-Drigalski agar.

A partial explanation may perhaps be found in the fact that some workers appear not to make use of dulcite, saccharose or salicin for the identification of "paratyphoid" bacilli, and may in some cases describe as *B. paratyphosus* (B) strains which would be excluded by others, who apply more extensive cultural tests. One strain sent to us as *B. paratyphosus* (B) differed widely in its cultural characters from standard strains of *B. paratyphosus* (B), and did not agglutinate (beyond a dilution of 1 in 40) with paratyphoid and *suipestifer* sera. Morgan (1907) has shown clearly that organisms occur with considerable frequency in the human intestine, which in some respects resemble "paratyphoid" bacilli, but which can be distinguished from them by adequate cultural and fermentation tests.

It is not improbable, however, that actual differences in the regional distribution of these organisms largely account for the apparently discordant observations of various writers. Although bacilli of the "paratyphoid" group have very rarely been isolated (apart from cases of paratyphoid fever) in London, Williams, Rundle and Murray (1910), working in Liverpool, obtained *B. suipestifer* from seven (probably seventeen) cases of summer diarrhoea. Again Sobernheim (1910) only occasionally found paratyphoid bacilli in Berlin, either in food or in healthy men, whereas Conradi (1909) frequently isolated these organisms from typhoid convalescents and healthy men in Western Germany.

Whether the distribution of these bacilli is such that in some districts they are frequently obtained both from food and from man, and in other districts they are rarely met with in food or in man, has yet to be proved. But the available evidence certainly suggests that such may be the case.

(*b*) *Examination of strains of the paratyphoid group.*

(1) *Cultural characters.*

All the strains gave the cultural reactions characteristic of the "paratyphoid" group of bacilli, except No. 20 (Lieblang), which fermented no sugars and was probably *B. faecalis alkaligenes.*

(2) *Agglutination reactions.*

Four sera were systematically employed for the carrying out of the agglutination tests, and in addition other sera were occasionally used.

The results, which are given in Table IV, show that with one or two exceptions all the strains were agglutinated equally well by the sera (A and B) obtained from rabbits, which had been injected with killed cultures of *B. suipestifer* and *B. paratyphosus* (B). In the case of the sera (C and D) obtained by the injection of living cultures into rabbits the agglutination limits were not the same for all strains and some indication was revealed of the existence of two types of bacilli. Some strains behaved like the standard strains of *B. suipestifer*, others like

TABLE IV. *Agglutination reactions of the strains.*

		Serum			
		From rabbits injected with killed cultures		From rabbits injected with living cultures	
		A	B	C	D
Organisms	Source	*B. suipestifer* Wassermann	*B. paratyphosus* (B) Schottmüller	*B. suipestifer*	*B. paratyphosus* (B) McWeeney
A. *Standard strains.*					
(*a*) *B. paratyphosus* (B)	Schottmüller (1901) original strain	4,000	2,000	5,000	20,000
(*b*) ,,	From Prof. McWeeney	4,000	2,000	5,000	20,000
(*c*) *B. suipestifer*	Laboratory strain	4,000	2,000	10,000	10,000
(*d*) ,,	From Prof. Uhlenhuth	4,000	2,000	10,000	5,000
(*e*) ,,	From Prof. Wassermann	—	—	10,000	10,000
B. *Strains investigated.*					
(1) Eisenmeyer	Paratyphoid fever case	2,000	2,000	5,000	20,000
(2) Bieber	"Paratyphoid" carrier (Dauerträger)	4,000	2,000	10,000	20,000
(3) Kunz	,, ,, ,,	2,000	1,000	5,000	20,000
(4) Vogt	,, ,, ,,	4,000	2,000	5,000	20,000
(5) Schnorr	,, ,, ,,	4,000	2,000	5,000	20,000
(6) Kant	Healthy man	4,000	2,000	5,000	20,000
(7) Ma	,, ,,	4,000	2,000	5,000	20,000
(8) Düsseldorf	Food-poisoning (patient)	4,000	100	10,000	< 400
(9) Heydr. Ali	"Sick man"	2,000	2,000	10,000	5,000
(10) Ba	Food	4,000	2,000	10,000	5,000
(11) Liver	Liver cheese (Leberkäse)	4,000	1,000	10,000	5,000
(12) Sausage	Blood sausage (Blutwurst)	4,000	2,000	10,000	5,000
(13) Murrow	Food-poisoning (patient)	4,000	100	10,000	< 200
(14) Savage, *c*	Paratyphoid fever case	4.000	2,000	5,000	20,000
(15) ,, *w*	,, ,,	—	—	5,000	20,000
(16) Lyons	Acute gastro-enteritis	2,000	1,000	< 1,000	< 200
(17) Winfield	Paratyphoid fever case	2,000	2,000	5,000	10,000
(18) Gill	Acute gastro-enteritis	4,000	2,000	10,000	20,000
(19) Zender	Healthy man	1,000	1,000	10,000	2,000
(20) Lieblang	Paratyphoid fever case	100	< 100	< 400	< 200
(21) Lister	Water supply	—	—	2,000	20,000
(22) Bacillus F	Acute gastro-enteritis	—	—	10,000	5,000
(23) Victoria	,, ,,	—	—	10,000	5,000
(24) Garton	,, ,,	—	—	10,000	5,000

standard strains of *B. paratyphosus* (B). It was also noticed in the case of serum D, that some strains were agglutinated into large clumps leaving a clear surrounding fluid, whereas other strains, even in such low dilution of the serum as 1 in 100, formed minute clumps, which had little or no tendency to settle and leave a clear fluid. The absorption method made it clear that with serum D and to a less degree with other sera, homologous bacilli tended to form large clumps, while heterologous bacilli formed small clumps. This difference has also been observed by Seligmann and Sobernheim (1910), although they did not attribute any significance to the observation.

As might be expected from its cultural characters, strain 20 (Lieblang) was not agglutinated by any of the sera in dilutions higher than 1 in 100. It has in fact been our experience that no bacillus which is not in all respects identical in cultural characters with *B. suipestifer* (or *B. paratyphosus* (B)) is ever agglutinated beyond dilutions of 1 in 100 by a *suipestifer* or *paratyphosus* (B) serum. We have however met with two organisms, *i.e.* Nos. 16 and 19, which although identical in cultural characters with "paratyphoid" bacilli agglutinated rather feebly with our sera as compared with standard strains of *B. suipestifer* and *B. paratyphosus* (B).

It may be added that none of our sera agglutinated *B. typhosus, B. enteritidis* Gaertner, or *B. paratyphosus* (B) in higher dilutions than 1 in 100.

(3) *Absorption tests.*

The absorption method was introduced by Castellani (1902) and was at first used mainly for the diagnosis of mixed infections. An obvious extension of the principle underlying the absorption method is that, if all the agglutinins formed in response to vaccination with a given organism are removed from the serum not only by it but also by some heterologous organism, the two organisms must be identical.

The method, if employed for a number of strains, is very laborious and in the case of the "paratyphoid" group of bacilli it can be used in a much simpler form. It has been shown that the members of this group of bacilli fall into two and only two classes, namely, one corresponding in all respects to *B. suipestifer* (or *aertryck*) and one corresponding to *B. paratyphosus* (B). It follows, therefore, that a serum which has been absorbed by the heterologous organism, is all that is needed for the identification of an unknown strain belonging to this group. Thus if a *paratyphosus* (B) serum is absorbed with *B. suipestifer* until the heterologous agglutinin is removed, it will still agglutinate *paratyphosus* (B) but not *B. suipestifer*, and by the use of such a serum it is perfectly simple to identify an unknown strain, since an organism which gives the correct cultural reactions and is agglutinated in high dilution by *paratyphosus* (B) or *suipestifer* sera, can only be *B. suipestifer* or *B. paratyphosus* (B), so far as is known. It was found unnecessary to absorb the sera with all the unknown organisms, and we have used almost entirely *B. paratyphosus* (B) sera absorbed with *B. suipestifer*, and *B. suipestifer* sera absorbed with *B. paratyphosus* (B). We have however checked our results for some strains by absorbing different sera with unknown strains according to the original method of Castellani.

The method of partial absorption was found to be very satisfactory and the results were concordant; no discrepancy was revealed when the sera were absorbed by unknown strains. It is desirable nevertheless to remove the heterologous agglutinin very completely since different strains vary slightly in the readiness with which they absorb agglutinin from, and are agglutinated by, a given serum. This variation represents nothing more than a certain individuality among members of the same group of bacilli, and is in no way comparable to the distinction between *B. suipestifer* and *B. paratyphosus* (B). The bacillus "F," recently described by Williams, Rundle and Murray (1910), is an interesting example of such individuality. These authors obtained from cases of summer diarrhoea seven strains, three of which were identical with *B. suipestifer*, whereas four gave anomalous absorption reactions, and were regarded by the writers as being intermediate between *B. suipestifer* and *B. paratyphosus* (B) and were called bacillus "F." Dr Williams very kindly furnished us with a strain of this organism, and also a serum homologous for it. We found that the bacillus gave the cultural tests characteristic of the "paratyphoid" group. Agglutination and absorption tests gave the following results, the organism being compared with recognised strains of *B. paratyphosus* (B) and *B. suipestifer*:

TABLE V. *Agglutination reaction. Macroscopic Method.*

	Organisms		
Serum	Bacillus F	Lab. strain *B. suipestifer*	*Paratyph.* (B) (McWeeney)
1. *B. suipestifer*	10,000	10,000	5,000
2. *B. paratyphosus* (B) (McWeeney)	5,000	10,000	20,000
3. Bacillus F	5,000	5,000	2,000
4. *B. enteritidis* Gaertner (titre 5,000)	<100	<100	<100

TABLE VI. *Absorption tests.*

Serum	Agglutination limits after absorption: Bacillus F	*B. suipestifer*	*Paratyph.* (B) (McWeeney)
1. *Bacillus F.*			
Original titre... ...	5,000	5,000	2,000
Abs. with Bacillus F ...	<200	<200	<200
,, *B. suipestifer* ...	<200	<200	<200
,, *B. paratyph.* (B)	5,000	5,000	<200
2. *B. suipestifer.*			
Original titre... ...	10,000	10,000	5,000
Abs. with *B. paratyph.* (B)	10,000	10,000	<200
,, Bacillus F ...	<50	<50	<50
3. *B. paratyphosus* (B).			
Original titre... ...	5,000	10,000	20,000
Abs. with *B. suipestifer* ...	<400	<400	10,000
,, Bacillus F ...	<400	<400	10,000

It is perfectly clear that Bacillus F is indistinguishable from recognised strains of *B. suipestifer* and that the slight differences noted by Williams, Rundle and Murray disappear when the sera are fairly completely absorbed and the agglutination limits are subsequently determined. These authors[1] have accepted the results of our observations upon Bacillus F and also our interpretation of the slightly different results which they obtained; and they now regard Bacillus F as identical with *B. suipestifer* and not as a separate strain.

The general results obtained by the absorption method are set forth in Tables VII—X[2]. Table VII shows the results obtained with recognised standard strains of the *B. suipestifer* and *B. paratyphosus* (B). The strains obtained for investigation have been arranged in three groups, corresponding to their mode of origin, so far as it is known.

Group I (Table VIII) includes all strains obtained from food or from outbreaks of illness, clinically resembling acute food-poisoning. Group II (Table IX) includes all the strains obtained from cases of paratyphoid fever, and from persistent paratyphoid carriers (Dauerträger). Group III (Table X) contains strains concerning which information is inadequate or absent.

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[1] Private communication kindly made by Dr Williams.

[2] Absorption tests were also made with the sera A and B; the results have been omitted since they were identical with those obtained with sera C and D, which are recorded in Tables VII—X.

With two exceptions, the strains included in Group I are identical with the standard strains of *B. suipestifer*. Strain No. 16 was obtained from a severe outbreak of food-poisoning caused apparently by infected goose-flesh. It has all the cultural characters of the "paratyphoid" group, but agglutinates very slightly with *suipestifer* and *paratyphosus* (B) sera. As far as they go, its agglutination reactions suggest that it is a strain of *B. suipestifer*, but it agglutinates so feebly that absorption tests were of no help in identifying it. This is the only strain we have found to be culturally identical with *B. suipestifer*, but which was agglutinated only slightly by *suipestifer* and *paratyphosus* (B) sera.

Strain No. 18 is extremely interesting. It was obtained from the faeces of patients suffering from acute gastro-enteritis, clinically resembling food-poisoning. Subsequent enquiry showed that the outbreak

TABLE VII. *Absorption test.*

Examination of standard strains.

	B. suipestifer serum. Agglutination titre before and after absorption with *B. paratyphosus* (B)		*B. paratyphosus* (B) serum. Agglutination titre before and after absorption with *B. suipestifer*	
Strains examined	Original titre	Titre after absorption	Original titre	Titre after absorption
(*a*) *B. paratyph.* (B); Schottmüller	5,000	<200	20,000	10,000
(*b*) ,, McWeeney...	5,000	<100	20,000	10,000
(*c*) *B. suipestifer*; laboratory strain	10,000	5,000	10,000	<200
(*d*) ,, Uhlenhuth ...	10,000	5,000	5,000	<200
(*e*) ,, Wassermann ...	10,000	5,000	10,000	<400

TABLE VIII. *Absorption test.*

GROUP I. Examination of strains obtained from food or from cases of acute gastro-enteritis.

	B. suipestifer serum. Agglutination titre before and after absorption with *B. paratyphosus* (B)		*B. paratyphosus* (B) serum. Agglutination titre before and after absorption with *B. suipestifer*	
Strains examined	Original titre	Titre after absorption	Original titre	Titre after absorption
(1) No. 10. Food	10,000	5,000	5,000	<400
(2) No. 11. ,, ...	10,000	5,000	5,000	<400
(3) No. 12. ,, ...	10,000	5,000	5,000	<400
(4) No. 13. Food poisoning ...	10,000	5,000	<200	<200
(5) No. 8. ,, ,, ...	10,000	5,000	<400	<400
(6) No. 18. Ac. gastro-enteritis	10,000	<200	20,000	10,000
(7) No. 22. ,, ,,	10,000	5,000	5,000	<400
(8) No. 23. ,, ,,	10,000	10,000	5,000	<200
(9) No. 24. ,, ,,	10,000	10,000	5,000	<200
(10) No. 16. ,, ,,	<1,000	<400	<200	—

TABLE IX. *Absorption test.*

Group II. Strains from cases of paratyphoid fever or persistent "paratyphoid" carriers.

			B. suipestifer serum. Agglutination limits before and after absorption with *B. paratyphosus* (B)		*B. paratyphosus* (B) serum. Agglutination limits before and after absorption with *B. suipestifer*	
	Strains examined		Original titre	Titre after absorption	Original titre	Titre after absorption
(1)	No. 1.	Paratyphoid fever...	5,000	<200	20,000	10,000
(2)	No. 2.	,, carrier	10,000	100	20,000	10,000
(3)	No. 3.	,, ,,	5,000	<400	20,000	10,000
(4)	No. 4.	,, ,,	5,000	<400	20,000	10,000
(5)	No. 5.	,, ,,	5,000	<200	20,000	10,000
(6)	No. 14.	,, fever	5,000	<400	20,000	10,000
(7)	No. 15.	,, ,,	5,000	<400	20,000	10,000
(8)	No. 17.	,, ,,	5,000	<400	10,000	10,000
(9)	No. 20.	,, ,,	<400	—	<200	—

TABLE X. *Absorption test.*

Group III. Strains whose origin and pathogenic relationship are unknown or doubtful.

			B. suipestifer serum. Agglutination limits before and after absorption with *B. paratyphosus* (B)		*B. paratyphosus* (B) serum. Agglutination titre before and after absorption with *B. suipestifer*	
	Strains examined		Original titre	Titre after absorption	Original titre	Titre after absorption
(1)	No. 6.	Healthy man ...	5,000	<400	20,000	10,000
(2)	No. 7.	,, ,, ...	5,000	<200	20,000	10,000
(3)	No. 19.	,, ,, ...	10,000	<100	5,000	<200
(4)	No. 9.	"Sick" man ...	10,000	10,000	5,000	<200
(5)	No. 21.	Water	2,000	<200	20,000	10,000

had not been caused by diseased food, but was possibly brought about by a human paratyphoid carrier, and that it is not a genuine example of food-poisoning.

All the strains included in Group II are identical with *B. paratyphosus* (B), except No. 20, which was not agglutinated by *suipestifer*, *paratyphosus* (B) (or Gaertner) sera, although its cultural characters were identical with those of *B. paratyphosus* (B).

No definite history of the strains included in Group III could be obtained. Three were from healthy men, but it was not known whether they were true paratyphoid carriers, or whether they had recently suffered from paratyphoid fever or food-poisoning. One was from a "sick" man, but the nature and causation of the illness could not be ascertained by us. Strain No. 21 was isolated from a well water which was otherwise bacteriologically satisfactory; no further information could be obtained with regard to it, except that attacks of jaundice had occurred among those who drank the water.

It thus appears that with two exceptions (Nos. 16 and 19) all the strains which we examined could be identified without difficulty by the absorption method as either *B. suipestifer* or *B. paratyphosus* (B). Every strain which was agglutinated by suipestifer and paratyphoid sera gave perfectly sharp and definite results when tested by the absorption method. The strains, which were obtained from undoubted cases of paratyphoid fever or persistent carriers, were all identical with standard strains of *B. paratyphosus* (B), while the organisms obtained from food or from outbreaks of illness definitely attributable to infection by food were identical with *B. suipestifer*.

Discussion of results.

There seems to be no doubt that those strains of bacilli which, by virtue of their cultural characters and agglutination reactions, belong to the paratyphoid group, can be further separated into two classes by means of the absorption method[1]. We regard the distinction which is revealed by this method as a real one, and we consider that, although they are closely related, *B. suipestifer* (with which *B. aertryck* is identical) and *B. paratyphosus* (B) are different organisms.

It has now been shown that *B. paratyphosus* (B) causes not only paratyphoid fever, but also sudden acute illness (Bainbridge and Dudfield, 1911); and the distinction formerly drawn on clinical grounds between infection by *B. suipestifer* and by *B. paratyphosus* (B) can no longer be maintained. Nevertheless, the distinction between these two organisms is of importance from an epidemiological point of view, since they appear to have a different distribution in nature.

Although we have not examined a very large number of strains, all the strains of *B. paratyphosus* (B) in our series were derived from man. We are disposed to regard the human alimentary canal (including the bile passages) as the normal habitat of *B. paratyphosus* (B) while admitting that paratyphoid "carriers" may cause infection not only directly but also indirectly by contaminating otherwise sound food, just as is the case with typhoid carriers. Under these circumstances it is to be expected that the bacilli may occasionally be found in food, polluted by a carrier, although we have not met with such an instance.

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[1] Dr H. R. Dean has examined a number of the strains of our series by the complement deviation method; his results confirm our observations and show that strains of *B. suipestifer* and *B. paratyphosus* (B) can be differentiated from one another. (Private communication.)

Lentz (1905) appears to have been the first to recognise the existence of persistent paratyphoid carriers; in 1905, he recorded five cases, one of whom had possibly given rise to a small epidemic of paratyphoid fever. During the investigation of a typhoid epidemic, Wernicke (1907) found a paratyphoid carrier, who was employed in a milk shop; and the evidence suggested that a number of cases of paratyphoid fever were caused by this carrier.

More recently, Prigge (1909) has described several persistent carriers, whose serum agglutinated paratyphoid bacilli, and who had either suffered from paratyphoid fever, or had been in contact with cases of paratyphoid fever. Strains Nos. 2, 3, 4, and 5, of our series were obtained from these cases. The frequency in carriers of disease of the bile-passages is also of interest. Hamilton (1910) examined twenty-four cases of chronic cholecystitis and found five carriers, and Gaethgens (1907) met with two carriers, who were suffering from chronic cholelithiasis; almost all these carriers were women. No paratyphoid carrier has yet been recorded in this country although the outbreak of illness, from which strain No. 18 was obtained, was thought, mainly on epidemiological grounds, to be caused by a carrier[1].

The part played by carriers in the causation of paratyphoid fever has still to be worked out, but it seems possible that careful search for carriers among those in actual contact with cases of paratyphoid fever may furnish a clue to the epidemiology of that disease.

In contrast to *B. paratyphosus* (B), it appears from our observations that the usual habitats of *B. suipestifer* are first the alimentary canal of the pig and perhaps other domestic animals, and, secondly, meat derived from such animals. No unimpeachable evidence has been brought forward as to the prevalence in this country of *B. suipestifer* in food or in the lower animals. The observations of Morgan[2] (1905), who obtained this organism by injecting into guinea-pigs mixed cultures from the faeces of pigs, are open to criticism, since Petrie and O'Brien (1910) have shown that it may often be obtained from healthy guineapigs; and there is other evidence that it may be obtained from guinea-pigs inoculated with material in which this bacillus was certainly not present.

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[1] A recent epidemic of gastro-enteritis at Wrexham is attributed to infection by a paratyphoid carrier. (See p. 89 this *Journal*. Ed.)

[2] Dr Morgan himself now regards the results of these experiments as inconclusive. (Private communication.)

In Germany, however, both Uhlenhuth (1908) and Seiffert (1909) have obtained *B. suipestifer* from the alimentary canal of the pig in a fair percentage of cases; and the strains Nos. 10, 11 and 12 of our series, which were isolated from apparently healthy food by German workers, were identical with standard strains of *B. suipestifer*.

Although the recent observations of Sobernheim (1910) and of Zwick and Weichel (1910) seem to show that bacilli of the "paratyphoid" group are much less often present in food (in some parts of Germany) than was at first supposed, there is no doubt of their occurrence in apparently healthy food. Under these circumstances they must, from time to time, be ingested by man, although their stay in the human alimentary canal is apparently very transitory. Thus Conradi (1909) found that when meat containing these bacilli was eaten by man, they could be obtained from the faeces on the following day, but not subsequently. Prigge (1909) also noticed that individuals who ate such meat, showed no evidence of infection, and that the transitory occurrence of bacilli of the "paratyphoid" group in their alimentary canal did not increase the agglutinating power of their blood for these bacilli. It may be noted that persistent *B. suipestifer* carriers have not been observed in man. If, as our observations lead us to suppose, these writers were dealing with strains of *B. suipestifer* it would appear that the isolation on a single occasion of *B. suipestifer* from the faeces of a healthy man, whose serum does not agglutinate the organism in higher dilutions than that of normal individuals, has no pathological significance.

Nevertheless, outbreaks of acute gastro-enteritis have occurred (Durham, 1898, and De Nobele, 1898) in which the epidemiological evidence pointed to infection by food, in which *B. suipestifer* was isolated both from the food and from the excretions or organs of the patients, and in which the serum of the patients agglutinated this organism in high dilutions. Such evidence that *B. suipestifer* is capable of causing acute infection cannot be disregarded, although the rôle of this organism in swine fever may arouse scepticism as to its capacity to initiate an infection. In swine fever and epizootics among guinea-pigs, it appears to be merely a secondary invader and not primarily a cause of the infection[1]; and it would be a matter of great interest if *B. suipestifer* should prove to occupy a similar subsidiary position in relation to "food-poisoning" outbreaks in man.

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[1] Uhlenhuth and Hübener (1908); Petrie and O'Brien (1910).

Further investigation is needed in order to account for the occurrence of this organism in man at one time as an apparently harmless saprophyte, at another in association with acute gastro-enteritis.

SUMMARY AND CONCLUSIONS.

1. The term "paratyphoid group of bacilli" has been confined in the present enquiry to those strains which in their cultural characters and agglutination reactions are indistinguishable from *B. suipestifer* and *B. paratyphosus* (B). *B. enteritidis* Gaertner and *B. paratyphosus* (A) are excluded.

2. The strains of bacilli belonging to the "paratyphoid" group can be separated into two classes by means of the absorption method, namely one group identical with recognised, standard strains of *B. suipestifer* and one identical with standard strains of *B. paratyphosus* (B); and we regard these two bacilli as separate organisms.

3. In our experience, *B. suipestifer* has been found only in food or in outbreaks of acute illness attributable to food-poisoning, whereas *B. paratyphosus* (B) has been found in cases of paratyphoid fever or in persistent paratyphoid "carriers."

4. We are disposed to put forward the suggestion indicated by these observations, that these two organisms have a different distribution in nature, the normal habitat of *B. suipestifer* being the alimentary canal of the pig (and other animals) and of food derived from such animals, whereas the normal habitat of *B. paratyphosus* (B) is the human alimentary tract (including the gall-bladder).

5. The examination of cultures from the faeces and urine of 300 typhoid convalescents was completely negative, neither *B. suipestifer* nor *B. paratyphosus* (B) being obtained. These observations confirm those of other writers, and, apart from carrier cases, the occurrence of *B. paratyphosus* (B) in healthy human beings appears to be unknown in this country.

Our thanks are due to Drs Fisher, Trautmann, Uhlenhuth, Williams, Rommeler, Prigge, Schern, Friedrichs, Savage and Fowler, who have very kindly supplied us with strains of bacilli for investigation.

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Brief Notes on the Structure and Development of Spirochæta anodontæ Keysselitz.

By

W. Cecil Bosanquet, M.D.

With Plate 15.

Much uncertainty still prevails as to the structure and biological relationships of the spirochætes. Earlier observers —not altogether influenced perhaps by a desire to prove their affinities with the hæmoflagellates—equipped them with an undulating membrane and a complicated nuclear apparatus, the existence of which is very problematical; and much controversy has existed as to their mode of division, longitudinal or transverse. The very claim of the organisms found in Mollusca, as well as that of the smaller spirochætes, to be called by this name, depending as it must on their relationship to Sp. plicatilis Ehrenberg, the type-species, is not very firmly established, since the structure of the latter—a system of large waves, each broken by smaller undulations superposed on them, with a central axial rod and a series of dots, regarded as nuclei—is markedly different from that of the later additions to the genus. It is desirable, therefore, to record any observations which may tend to throw light on the morphology and life-history of these organisms.

My own observations have been carried out on Sp. anodontæ, a species easily obtained, and one which may be regarded as fairly typical of the spirochætes which are parasitic in Mollusca.

With regard to the so-called undulating membrane of

Sp. anodontæ I am inclined to agree with Schellack, who holds that the appearance which has been thus interpreted is an artifact, due to splitting of periplast, since I found that the better my preparations were fixed, the fewer were the examples of this condition. It was found almost invariably in films dried in the air (fig. 1), but very exceptionally in those fixed in osmic vapour. It seems possible that the periplast, if it is so to be called, adheres to the slide in the position in which death of the spirochæte occurs, and that subsequently the protoplasm of the organism shrinks and thus straightens its outline a little, leaving a line of periplast following a different line from that finally adopted. At other times the periplast seems to shrink most and to form a band uniting the cells of the spirochæte (fig. 2).

I have seen a considerable number of specimens which exhibit a dark line running along one side (figs. 3, 4). This probably corresponds with the "crest" described by Gross. Judging from the illustrations of spirochætes in section given by him and previously by Fantham, it would seem that the organism possesses a sheath which is loose enough to form a fold along one side in certain conditions. The sheath or periplast stains more darkly than the body-substance of the spirochæte, and hence the double layer appears as a dark line. Splitting of the sheath into fibrillæ seems sometimes to occur, as in fig. 6.

On the other hand, I find it difficult to agree with Schellack that two separate species of spirochæte are present together in Anodonta, one having blunt (figs. 1, 3, 4) and the other sharp extremities (figs. 7, 8, 9). The amount of difference seen is scarcely sufficient to suggest a specific differentiation. Further, the great variation in the length and thickness of the individuals in each class thus formed is such as to negative the possibility of distinguishing species by length, as he further suggests. It is noteworthy that Schellack distinguishes two species of spirochæte in more than one kind of mollusc, and that Gross also describes two, very similarly differentiated, in Pecten jacobæus. This rather strongly suggests that the same spirochæte may present different shapes and sizes. I have never seen specimens of Sp. anodontæ with transverse lines near the middle of the length of the organism, pointing to transverse division by means of a septum here formed, as is depicted by Schellack.

As to the actual method of division, I had the opportunity of observing an instance of what appeared to be longitudinal fission. This was in a preparation containing living spirochætes in a drop of fluid from the mantle-cavity of the mussel. My attention was attracted to an individual which remained fixed at one point, near to the edge of the preparation, and wriggled feebly, instead of dashing about like the others. I then noticed that this spirochæte seemed to possess two "tails," if this expression is allowable (fig. 11*a*); and on watching it I noticed that a line, dark at one moment, refringent at another, as the creature moved, was gradually extending along the body of the spirochæte from the point of bifurcation towards the opposite extremity. Fission proceeded along this line, but not quite regularly as in tearing a strip of calico, since at one moment there was visible a sort of loop in the "anterior" part of the organism—using this term for the portion which remained single longest—before the process of division had extended quite to this point from "behind" (figs. 11*b*, *c*). When the longitudinal fissure had extended nearly to the "anterior" end, the organism suddenly jerked itself away out of the field of the microscope and I was unable to see the final separation of the daughter, individuals; but as I was unable, on searching through the preparation, to find the dividing spirochæte again, I could only conclude that the separation took place almost immediately, the two resulting spirochætes being then indistinguishable from the other individuals present.

A possible source of error in this observation lies in the slenderness of the organisms and in the difficulty of accurately observing them during movement. It is scarcely possible to exclude the explanation that an organism may become folded upon itself as a preliminary to undergoing transverse fission (figs. 12, 13). This alternative possibility was strongly brought to my mind by a preparation in which many living spirochætes appeared to have loops at one extremity, while they revolved rapidly on a longitudinal axis. As their movements slackened, these organisms appeared to have a shape which might be compared with that of a hair-pin having its "legs" twisted together. On fixing and staining this preparation, specimens were found both of the condition just described (fig. 14), and also of what seemed to be a succeeding stage, in which division had taken place at the bend of the loop (fig. 15). I could find no instance of a spirochæte simply looped at one extremity, as might have been expected to occur if longitudinal division took place, starting at a point a little distant from the extremity of the spirochæte. I was at first tempted to regard the pairs of closely apposed organisms here seen as being instances of conjugation, but the explanation just given seems more probable in view of the looped forms seen alive in the preparation. Division by "incurvation" is stated by Gross to occur in the spirochæte present in Pecten jacobæus (called by him Cristispira pectinis), and he believes it to be the characteristic of all spirochætes. Nuttall, Fantham and Porter state that in the small spirochætes both transverse and longitudinal division occurs. If this be so, it would be interesting to ascertain whether any difference in environment is responsible for their adoption of each method respectively, or whether the two processes occur at different points in the life-cycle.

With regard to the internal structure of Sp. anodontæ, I have always found that specimens taken from the crystalline style of the mussel stain homogeneously like Bacteria (fig. 7). Those, however, which are present in the stomach of the mollusc, especially after the style has dissolved, show beading of the substance of the organism, as is depicted by Keysselitz in his original memoir (figs. 16, 17, 18). Since the style is apparently the most favourable medium for the life of the spirochæte, I am inclined to look upon the beaded forms as either involutionary or developmental. The following observation supports the view that the change is, in some cases at least, one of development.

A piece of crystalline style was dissolved in tap-water and sealed up with vaseline for forty-eight hours under a cover-slip on a slide. At the end of this period the cover was removed, and the preparation was fixed in osmic vapour and stained with thionine. The spirochætes, which had originally been very numerous, were found to have almost entirely disappeared, while their place was taken by elongated, variously curved bodies, closely corresponding with them in length and thickness, containing irregular masses of staining substance (figs. 19 and 20). Some definite spirochætes present showed beading of protoplasm and separation into segments (segmentation, plasmolysis?), and it was possible to trace a fairly definite series of gradations between such spirochætes, as seen in this and other preparations, and strings of bead-like or coccoid bodies, which seemed to constitute the final stage of development (figs. 16, 18, 21, 22). It seems then that Sp. anodontæ goes through a stage in development in which it breaks up into coccoid bodies, just as, according to Leishman, Sp. duttoni breaks up into similar bodies inside the body of the tick. If this be so we have, on the one hand interesting evidence of the generic unity of the small and large spirochætes, and on the other, of the affinity of the whole group with the Bacteria rather than with the Protozoa.

The portion of style used for the above observation was taken from a mussel which had been kept at a temperature just above freezing-point. The spirochætes seemed specially numerous and lively at this temperature, and dividing forms were numerous. I also found some organisms in the water in which the mussels were kept. Most of these were stiff and motionless, but one or two individuals were actively moving when first seen. The addition of water to a piece of the style seems to have the effect of rendering a large number of the organisms motionless and rigid (fig. 23), and it is tempting to speculate as to whether the difference between what has been called a Treponema, in which the curls are more or less rigid, and a Spirochæte, in which there is more flexibility, may not be dependent to some extent on the medium in which the organism is examined. Accompanying the spirochætes in the water were a considerable number of spirillar organisms, exactly resembling them in microscopical structure (figs. 23, 24 *a*, *b*). It seems possible that the segmenting spirochætes (fig. 19) may divide first into short spirilla in some instances before undergoing development into the coccoid stage. At a low temperature the style of the mussel is more slowly dissolved than at the temperature of the room. I have found that, as a rule, this body entirely disappears in about a week or ten days after the mussel is taken up from its native place. One mussel, which was kept for three weeks in an aquarium, where presumably food was plentiful, formed a new style, so far as could be judged, since all the other mussels of the same batch had lost theirs long before. This re-formed style was quite free of spirochætes.

My best thanks are due to Prof. Minchin for allowing me to use his laboratory, and for his advice and encouragement; also to Miss Rhodes, who has drawn the illustrations to this paper.

LISTER INSTITUTE,
November 4th, 1910.

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EXPLANATION OF PLATE 15,

Illustrating Dr. W. Cecil Bosanquet's paper on "Brief Notes on the Structure and Development of Spirochæta anodontæ Keysselitz."

[All the figures, except Figs. 1 and 11, are from specimens fixed in osmic vapour and stained with thionin, and all are drawn at a uniform magnification of 2000.]

Fig. 1.—Spirochæta anodontæ, showing so-called undulating membrane. From a film dried in the air and stained with iron-hæmatoxylin.

Fig. 2.—Sp. anodontæ—long thin specimen from crystalline style.

Fig. 3.—Same. Long thick specimen.

Fig. 4.—Same. Short thick specimen.

Fig. 5.—Same. Short thin specimen.

Fig. 6.—Same, showing splitting of (?) sheath.

Fig. 7.—Same. Slender specimen showing uniformity of structure and absence of "undulating membrane," like a bacterium.

Fig. 8.—Same. Short specimen with pointed ends.

Fig. 9.—Same. Longer specimen with pointed ends and somewhat stiff curls (not well shown in the figure).

Fig. 10.—Same. Short thin specimen with apparent undulating membrane.

Fig. 11.—*a*, *b*, *c*, stages in apparent longitudinal division of Sp. anodontæ (diagrammatic).

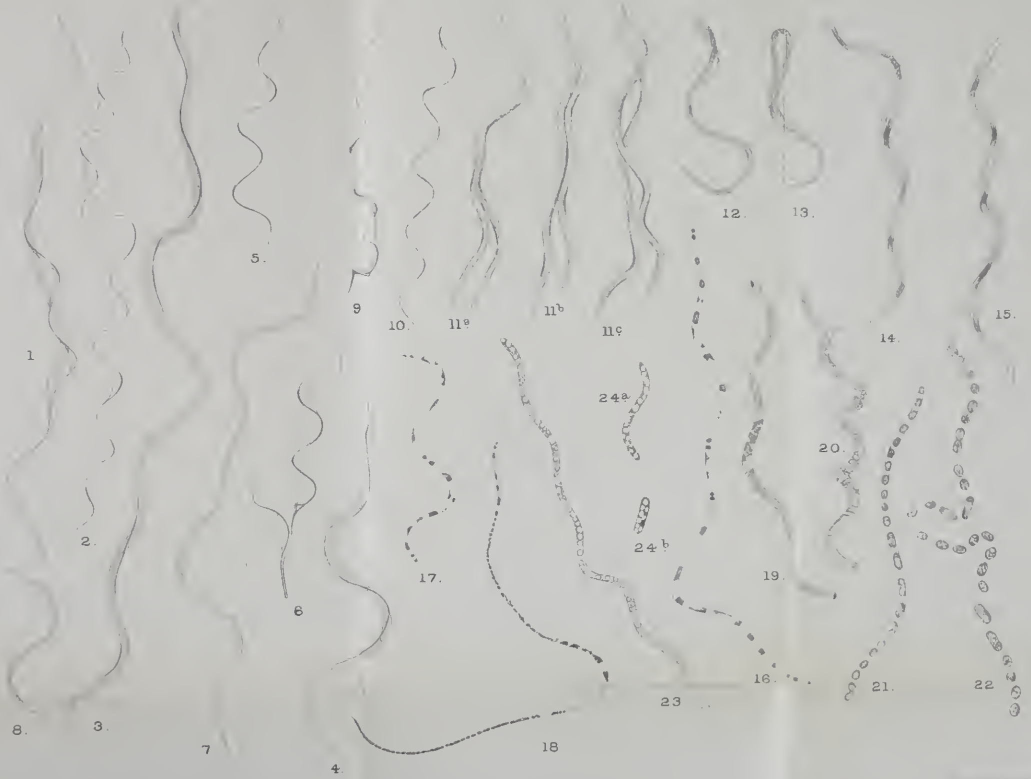
Figs. 12, 13.—Stages in "incurvation" (?) of Sp. anodontæ.

Figs. 14, 15.—Complete incurvation (?) and subsequent transverse division.

Figs. 16, 17, 18.—Sp. anodontæ, from the alimentary canal of the mussel, showing plasmolysis (?).

Figs. 19, 20, 21, 22.—Apparent development of spirochætes into coccoid bodies. From a preparation of crystalline style dissolved in water and kept for forty-eight hours.

Fig. 23.—Spirochæte from water in which mussels had been kept, for comparison of structure with figs. 24 *a*, *b*, showing spirilla from same water.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OBSERVATIONS ON LEUCOPROTEASE AND "ANTI-LEUCOPROTEASE."

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2 Figures.

According to Kossel (1888) Fritz Müller discovered that phthisical sputum digests fibrin and coagulated albumen in weakly alkaline media, but the fact that antiseptic pus digests proteins seems to have been first observed by Leber.

Achalme (1899) separated proteolytic ferments from pus from various sources and Erben (1903) and Schumm (1904) drew attention to the notable quantity of albumoses in blood from cases of myologenous leukaemia which had been incubated, and the relative absence of this proteolysis in the incubated blood from cases of lymphatic leukaemia. Ascoli and Mareschi (1901) showed that the peritoneal exudate produced by aleuronat underwent autolysis under aseptic conditions.

More recently the subject of a proteolytic ferment in leucocytes has been studied by a number of authors amongst which the principal are Opie (1905 and 1906), Müller and Jochmann (1906 i. and ii.), Jochmann and Lockermann (1908), and Fiessinger and Marie (1909).

All agree as to the presence of a tryptic ferment in the polynuclears but the existence of another protease acting in feebly acid solutions in lymphocytes described by Opie is questioned by Müller and Jochmann and Fiessinger and Marie.

Opie working with sterile exudates obtained by injection of aleuronat into pleural cavities of dogs, as well as with pus from turpentine-produced abscesses in various animals, found that in the leucocytes collected from such artificially produced exudates by centrifugalisation two ferments may be demonstrated. The main point of the proof of the separateness of these ferments is that in an alcohol ether dried preparation only one remains. He calls the two ferments *lymphoprotease* and *leucoprotease.*

Lymphoprotease is associated with mononuclear leucocytes and resembles pepsin in acting at low degrees of acidity but it is not pepsin as it fails to act in 0·2 % HCl though it will do so in feebler dilutions. It is inhibited by alkalinity but not by the serum as such.

Leucoprotease is associated with the polymorpho-nucleated cells and is a tryptic-like ferment acting best in faintly alkaline but well also in neutral media; Opie does not think this is trypsin for it is very weak compared with the latter. It is held in check by normal sera. It is not destroyed by treatment with alcohol and ether and drying. The various results of Opie and others with regard to other points will be found in their respective sections.

Lymphoprotease was also obtained by Opie and Barker (1907) by injecting tubercle bacilli into the pleural cavity of dogs. It has been previously indicated that its origin from lymphocytes has been disputed but as my own experiments deal only with leucoprotease from human pus the question will not be entered into.

I was anxious to inquire into certain points as regards the working of the so-called "anti-body" manifested in normal sera, and especially to see if one could increase this anti-proteolytic power by immunisation and if there could be found any evidence in favour of or against the idea that any anti-effect is due to a true anti-body similar to those concerned in production of immunity to various toxins and so forth. This work is not intended to be complete—the subject is in itself rather complicated and it has been difficult for me even with such facilities as I have had at the Lister Institute to get sufficient material—hence I have merely been able to cover part of the ground, the remainder of which I hope to attempt on a future occasion. Two difficulties which I have found to render accurate work on certain points so far almost impossible are, firstly the ferment in my preparations is very weak, and secondly it is always accompanied by large amounts of proteid, and I have not so far been able to devise a satisfactory method, that is to say necessarily a conservative method, by which I can at all purify the preparation.

Methods.

The material I have examined was derived from thirteen specimens of empyema pus. In the majority of experiments the material was, after screening off as much blood clot, fibrin, etc. as possible, centrifuged and washed with normal saline three or four times. The final debris was shaken well with methylated spirit, this either filtered or centrifuged, and pipetted off and then absolute alcohol and finally alcohol-free dried ether used in the same way. The debris from this process was collected in flat trays, fanned vigorously for a while and powdered, then placed in a desiccator, till apparently completely dry, if necessary re-powdered, and then kept in the desiccator till required. Here apparently it is little affected as regards its activity by lapse of time. The final product is a very fine dry powder which has a colour varying from white to pink, according to the amount of blood clot present.

For estimating the activity of the enzyme the following process, which is similar to that used by Hedin in his experiments with trypsin, was employed. This only differs in detail and the form of precipitation of the proteid from that of Opie. Measured amounts of ferment preparation, either by weighing quantities dried of the powder or pipetting suspensions or extracts, were taken into suitable stoppered bottles of about 150 c.c. capacity. To this was added the substrate used, either 25 % boiled solution of casein with toluene, or boiled fibrin, or gelatin, or egg albumen. In testing the effect of serum this was also added. Controls were made with the enzyme preparation heated in moist condition above 90° C. for several minutes. To each flask a definite amount of toluene was added as preservative and the flasks well shaken and incubated in a hot room, where they were kept in motion by an electric shaker. At the end of the digestion period the flasks were taken off, cooled to the room temperature, and precipitated by equal amounts of the tannic acid solution. The mixture was well shaken and filtered. The filter papers should be all of one kind and the same size, as also should be the filter funnels. The temperature at which the precipitation was carried out seemed to be important, as also did the time the materials were left in contact before filtering. A feature of interest noticed was that a perfectly clear filtrate, which subsequent estimation proved to contain large amounts of proteid, usually shows after some minutes a faint cloudiness, very hard to separate. This does not occur in the control flasks and the amount of proteid in this precipitate is negligible, as whether one refilters to clearness or not, the results were within my experimental error.

Aliquot parts of these filtrates were Kjeldahled in the usual way and the ammonia estimated. The index of activity is furnished by the amount of ammonia in the distillates. The figures are expressed as ammonia represented by c.c. N/10 acid.

The potency of my preparations was not great and the following two experiments which are given in detail illustrate the order of activity obtained.

Powder No. 4. Time of digestion 5 days. Substrate casein 2·5 % in 0·25 % sodium carbonate solution.

			Nitrogen as c.c. of deci-normal sulph. acid in 100 c.c. of filtrate
A.	Substrate 100 c.c. + powder 0·04 gm.	=	16·9
B.	Substrate 100 c.c. + powder 0·04 gm. (heated)	=	4·1

Each flask precipitated with 100 c.c. tannic acid solution. 100 c.c. of filtrate taken for estimation.

Powder No. 6. Time of digestion 5 days. Substrate casein 2·5 % in 0·25 % sodium carbonate solution.

			Nitrogen as c.c. of deci-normal sulph. acid in 50 c.c. of filtrate
A.	Substrate 50 c.c. + powder 0·4 gm.	=	30·6
B.	Substrate 50 c.c. + powder 0·4 gm. (heated)	=	3·0

Each flask precipitated with 50 c.c. tannic acid solution. 50 c.c. of filtrate taken for estimation.

Influence of Amount of Enzyme upon the Rate of Action.

The following experiments were undertaken with a view to inquire into the rate of change brought about by the enzyme in the alcohol ether dried preparation. Bottles containing varying amounts of the powder with equal amounts of substrate were allowed to digest for varying lengths of time. In the first chart the abscissa represents time in days and the vertical ordinates thereto activity measured as cubic centimetres of deci-normal sulphuric acid. There are four curves of which the lowest represents the results obtained by allowing 0·05 gram of the powder to act on equal amounts of substrate for varying times. Four bottles containing identical amounts of substrate and powder were put into the hot room at the same time. One of these was taken out on the expiration of the times mentioned in the chart and the nitrogen as ammonia in the tannic acid filtrate estimated. The other three curves similarly represent activity at varying times of 0·1 gram, 0·2 gram and

0·4 gram respectively. They were obtained in the same way except that the curve for 0·2 gram was not continued so long and the estimations were made at different times.

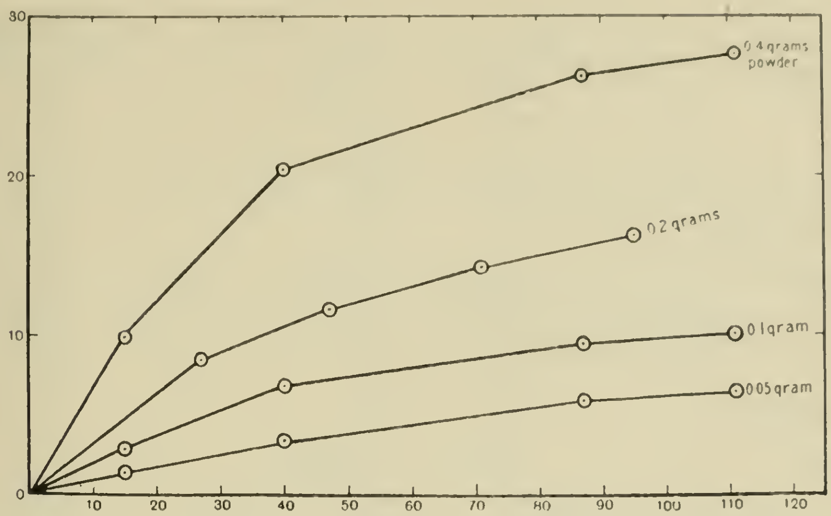

Chart 1.

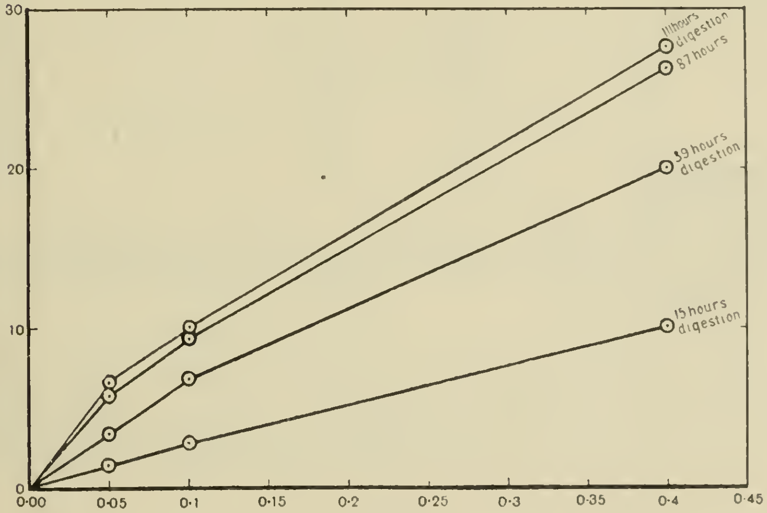

Chart 2.

The second chart is compiled from the same figures and shows certain points more clearly. In it the abscissa represents the amounts of enzyme while the vertical ordinates represent activity as cubic centimetres of deci-normal acid representing ammonia in filtrate. There are four curves at 15, 39, 87, 111 hours respectively showing effect of increasing amounts of enzyme.

The figures following the charts are the protocols of experiments from which the charts are compiled.

Experiment 5.

Powder No. 6. Time 2½ days. Substrate, casein 2·5 $^0/_0$ sol. in sodium carbonate solution 0·25 $^0/_0$.

Powder		Casein sol.	Time		Nitrogen as c.c. of deci-normal sulph. acid in 50 c.c. of filtrate
0·05 gm.	+	50 c.c.	15 hrs.	=	4·4
0·05	+	50	39	=	6·4
0·05	+	50	87	=	8·8
0·05	+	50	111	=	9·4
0·05 (heated)	+	50	41	=	3·0
0·1 gm.	+	50 c.c.	15 hrs.	=	5·9
0·1	+	50	39	=	9·8
0·1	+	50	87	=	12·5
0·1	+	50	111	=	13·0
0·1 (heated)	+	50	111	=	3·0
0·2 gm.	+	50 c.c.	27 hrs.	=	11·6
0·2	+	50	47	=	14·5
0·2	+	50	71	=	17·1
0·2	+	50	95	=	19·2
0·2 (heated)	+	50	47	=	3·0
0·4 gm.	+	50 c.c.	15 hrs.	=	12·9
0·4	+	50	39	=	23·2
0·4	+	50	87	=	29·3
0·4	+	50	111	=	30·6
0·4 (heated)	+	50	111	=	? about 3

As precipitant 50 c.c. tannic acid sol. was used, 50 c.c. of the filtrate after this addition was taken in each case. The flasks were well shaken during the first ten hours and for short spells afterwards.

The points brought out by the above results with regard to the action of the enzyme under the described conditions, *i.e.* acting in closed flasks on a large excess of proteid may be summarised as follows:

Influence of amount of enzyme time constant. Chart 1.

1. There is an increase in the total action with the time up to the point of termination in these experiments. From the charts it seems that the maximum was nearly but not quite reached.

2. The amount of change per unit time is relatively greater in the earlier hours, falling off with the progress of time.

3. In the early hours and with small doses the rate of change is nearly proportional to the time.

Influence of amount of enzyme time constant. Chart 2.

1. The total action increases with the amount of enzyme (even the largest dose in these experiments is small relatively to the total proteid to be changed, as similar experiments with 0·8 gram showed a great increase of action).

2. With amounts of enzyme up to 0·1 gram per 50 c.c. and digestion time not exceeding 39 hours the rate of hydrolysis is proportional to enzyme added.

3. On increasing the amount of enzyme or the interval for digestion the rate falls more and more short of direct proportionality.

Effect of Reaction.

The following experiments show the effect of reaction on the activity of the powder.

Experiment 6.

Powder No. 3. Substrate, fibrin 0·5 gm.

			Nitrogen as c.c. of deci-normal sulph. acid in 20 c.c. of filtrate
A.	Boiled fibrin 0·5 gm. + powder 0·02 gm. + acetic acid 0·2 % 15 c.c. ...	=	0·8
B.	Boiled fibrin 0·5 gm. + powder 0·02 gm. + sodium carbonate sol. 0·25 % 15 c.c.	=	2·0
C.	Boiled fibrin 0·5 gm. + powder 0·02 gm. + water 15 c.c.	=	1·15
D.	Boiled fibrin 0·5 gm. + powder 0·02 gm. + water 15 c.c. } (boiled)	=	0·5

Added 15 c.c. tannic acid solution. Took 2 c.c. of filtrate for estimation.

This experiment displays the absence of action in weakly acid media while there is action in approximately neutral or alkaline media.

The following experiment conducted by a different method also gives us the same results. Here the experiment is merely qualitative. Small and equal quantities of the powder were allowed to act on gelatin at incubator temperature (37° C.) under the conditions of reactions shown, and examined as to power of solidifying by cooling in running water.

Experiment 7.

Powder No. 4*a* was used and toluene as antiseptic. These were added to a large test tube with 2½ inches of 10 % gelatin.

Reaction	14 hrs.	38 hrs.
Just alkaline to Lachmoid	fluid	fluid
Just acid to Lachmoid	solid	solid
Neutral to Lachmoid	fluid	fluid

Another qualitative experiment shows the same results. Here the method employed as a test of activity was the biuret test. Equal amounts of the powder No. 6 were allowed to act on boiled dried fibrin powder in the presence of acid alkali and neutral diluents. After 24 hours they were examined with the following results:

Experiment 8.

		Biuret reaction
1.	Powder + fibrin + acetic acid 0·2 %	–
2.	Fibrin + acetic acid 0·2 %	–
3.	Powder + fibrin + sod. carb. sol. 0·25 %	+ +
4.	Fibrin + sod. carb. sol. 0·25 %	–
5.	Powder + fibrin + normal saline	+ +
6.	Fibrin + normal saline	–

N.B. A solution of the powder itself does not give the reaction.

The above results show that the enzyme contained in the dried preparation from human pus acts best in weak alkaline solutions, also well in approximately neutral solutions but not in the presence of 0·20 % acetic acid.

Effect of Heat.

The following experiments were performed to show the effect of heat on the enzyme, *i.e.* to determine the point at which the enzymotic activity is no longer manifested. On investigation it becomes apparent in all such research that a factor equally important to the temperature to which the substance is raised, is the time taken to do so. It is not long before one realises the futility of saying that the death point is such and such a temperature. This will be found to differ considerably with the method used. If for instance the dried powder is simply heated in that dry condition in a hot air stove, it will resist for many minutes, up to a quarter of an hour at least, a temperature well over 100° C. Whereas, if the powder is moistened, it is killed at a far lower point; but besides this the time taken in heating is very important. Conditions are entirely different if we start heating the ferment from room temperature and bring it up to the required point than if we drop the ferment into a fluid heated to that point. Likewise if we keep the ferment less or greater time at any point so the result will differ. Two methods which I have tried give results showing what I mean, in Experiment 9 the heating was done in similar test tubes in a water bath already heated to the required amount. In this series the ferment is markedly affected by one minute at 65° to 67° C., that is to say one minute after the ferment reached 65° C. In Experiment 10, where the ferment is merely dropped into the water when that has reached the required temperature and is retained there the requisite time, it is not much affected by one minute between 69° and 71° C.

Other considerations, too, render this question I think one which gives very unprofitable results unless some standard method be adopted by all observers. Opie (1906 (1)) gives 67° C. as the thermal destruction point; but unfortunately gives little information as to his method. When it is an obvious fact that such factors as amount of proteid in solution, rate of rise of temperature, and others such as are stated above, so affect the results, it is hardly likely that any two observers will agree. Here I merely give the results of a few experiments which were really done as a means to know how I could most effectively kill the enzyme for controls.

Experiment 9.

Method. The heating was done by dropping weighed amounts of powder No. 9 into 20 c.c. of water raised to the required temperature in a water bath, taken off as required and cooled quickly. The 20 c.c. of water were heated in the bottles used for digestion and subsequently 50 c.c. of casein solution were added with 5 c.c. of toluene to each flask. After two days' digestion 50 c.c. of tannic acid solution were added and 50 c.c. of the filtrate from this taken for estimation. The figures represent nitrogen as deci-normal sulph. acid in 50 c.c. of the filtrate.

Heated to 62° C. and cooled immediately = 12·8.
,, 65° C. ,, ,, = 12·8.
,, 70° C. ,, ,, = 12·7.
,, 75° C. ,, ,, = 10·9.
,, 80° C. ,, ,, = 6·7.

Heated for one minute between 69 —71° C. = 12·2.
,, ,, ,, 69·5—71·5° C. = 12·15.
,, ,, ,, 72 —73° C. = 11·55.
,, ,, ,, 74·5—75° C. = 6·45.

Experiment 10.

Method. 0·2 grams of powder No. 5 were heated in 0·25 $^0/_0$ sod. carb. sol. to various temperatures and kept at these temperatures for one minute. Then to each flask 20 c.c. of 10 $^0/_0$ gelatin were added and the flasks put in the hot room (37° C.) to digest. Observations were made as to digestion by cooling the flasks under the water tap for the same time and noting the state of the gelatin.

	After 4 days	After 6 days
Powder not heated	fluid	fluid
1 minute at 65—67° C.	semi-solid	fluid
½ minute at 70—73° C.	solid	solid
1 minute at 73—75° C.	solid	solid
1 minute at 84—85° C.	solid	solid

Experiment 11.

Method. Powder was heated *dry* in watch glasses in a hot water jacketed stove to a temperature varying between 88—92° C. Then 0·1 gm. was allowed to act for three days on 15 c.c. 10 $^0/_0$ gelatin with 15 c.c. sod. carb. sol. 0·25 $^0/_0$.

	Time left in stove	Nitrogen as deci-normal acid 20 c.c. of filtrate
A.	0	2·9
B.	5 minutes	3·0
C.	10 ,,	3·1
D.	15 ,,	3·3
E.	20 ,,	3·2
Control heated at 110—120° C. for several minutes in hot air stove		1·3

40 c.c. tannic acid added. 20 c.c. of filtrate used for estimation.

The Anti-Effect of Sera.

The anti-tryptic action of normal serum was noted by Hahn (1897) and Achalme (1901). Achalme succeeded in increasing the anti-tryptic effect of guinea-pig serum by immunisation. Hedin (1905 and 1906 I.) has very fully investigated the neutralising action of normal serum upon trypsin. This effect he finds is larger if the serum be added to the trypsin before adding the substrate and there is an increase in anti-action, up to a certain point, with the time for which they are left together before adding the substrate. The combination occurs more readily the higher the temperature, up to a certain point. The amount of trypsin neutralised is independent of dilution. The anti-power of the serum can be completely neutralised, but trypsin cannot be completely rendered inert by serum. The amount of trypsin neutralised is greater relatively for small amounts than for large amounts of serum. The anti-body is completely destroyed by 0·1 to 0·2 °/₀ of acetic acid at 37° C. for 8 hours and is markedly affected by acid in a very short time. He found it impossible to separate anti-body and enzyme in a neutral mixture, *i.e.* to free the trypsin. In a further paper Hedin (1906 II.) compared the results obtained with serum with those obtained by the addition of charcoal to a tryptic digest. He found that this substance had a powerful effect in neutralising trypsin and that the neutralisation probably consisted of two consecutive stages. Firstly, a taking up or absorption of trypsin: in this stage the trypsin can be freed by the addition of more substrate. Secondly, a stage in which the trypsin is fixed. The amount fixed is larger the more charcoal used, the higher the temperature and the longer the time of interaction. He found this effect of charcoal to agree in every point as far as he could see with the effect of serum and concluded the latter to be a similar phenomenon.

The anti-tryptic action of normal sera was found by Landsteiner (1900) to be associated with the albumin fraction of the serum (Pick). This was confirmed by Cathcart (1904). Opie (1905, 1906) and Opie and Barker (1907), working with leucoprotease, found that serum either from purulent exudates or from blood inhibits the proteolytic effect of leucoprotease. This property is destroyed by acids and by heating to 75° C. for half an hour. A certain amount of serum is only capable of controlling a limited amount of enzyme. As in the case for trypsin the inhibiting action was associated with the albumin fraction of the serum and not with the globulin.

Müller and Jochmann (1906) also made observations on the anti-effect of serum. The anti-leucoprotease is not specific. The serum of one animal is equally effective against leucoprotease from all sources (Opie and Barker 1907).

The anti-body in normal sera neutralises both trypsin and leucoprotease (Jochmann and Lockermann 1908).

Variations in the anti-tryptic power of human serum have been found to occur in patients suffering from diseases involving cell destruction (Bittorf 1907) or associated with leucocytosis (Wiens 1907), tuberculosis (Wiens) and cancer (Brieger and Trebing 1908). Eisner (1909), however, finds that it is only in the cachectic state of cancer that the anti-tryptic power is modified.

In this country the subject has been exploited with a view to its possible utility in diagnosis by Golla (1909), Hort (1909) and Bayly

(1909). Golla has introduced means of considerable accuracy for measuring the anti-tryptic power of different sera under clinical conditions.

Before detailing my experiments with sera it will be useful to say a few words as to methods.

So as to make the total amount of proteid equal in each case, it was necessary in performing experiments with different amounts of fresh serum to make up the total serum content to the same figure by the addition of heated serum. Heated serum must also be used for maximum and minimum control. By suitable dilution we can arrive at a satisfactory method of getting a serum sufficiently heated and still in a liquid state. For this purpose I have found the dilution of 1 serum to 4 water the best, 1 serum to 4 normal saline is likely at times to precipitate before a sufficient temperature for use in controls is reached. For heating purposes I used a water bath already boiling; in this the dilute serum is immersed in a thin flask and kept moving briskly until the temperature as registered by a thermometer inside the flask reaches the required point. I have employed 90° C. as a useful temperature for heating all controls from the experience of such an experiment as No. 12. With regard to the effect of heat on the anti-power of serum, Opie found that heating equal quantities of serum diluted with equal amounts of salt solution to 75° C. for half an hour sufficed to destroy the anti-power. Lower temperatures seemed to afford a slight increase in the anti-power; he does not state how the heating was done. In the following experiment equal quantities, 10 c.c. of serum in the dilution of 1 serum to 4 water, were heated in the water bath from the water supply temperature to the required temperature in test tubes of equal thickness and calibre. Thus the time taken in heating to the required temperature should be in each case the same. The test tubes were immediately cooled under the water tap. This heated serum was allowed to act on a mixture of enzyme and substrate. In this case a fluid preparation $\frac{1}{2}\,^0/_0$ of the powder in 0·25 $^0/_0$ sod. carb. sol. was used in a dose of 20 c.c. in each flask. Of the casein substrate 50 c.c. were used in each flask. After $2\frac{1}{2}$ days' incubation 50 c.c. of tannic acid solution were added as precipitant and 50 c.c. of the filtrate from this taken for estimation. The results below show that it has begun to be affected at 72° C. and apparently is not much more affected by 76° C. But at 90° C. for several minutes it is completely destroyed so that this is a sufficient temperature to heat the serum for the controls.

Experiment 12.

Powder No. 5.

					Nitrogen as deci-normal acid in 50 c.c. of filtrate
With fresh serum				... ...	3·05
With serum heated to			50° C.	... ...	3·15
,,	,,	,,	55	... ...	3·2
,,	,,	,,	60	... ...	3·0
,,	,,	,,	70	... ...	3·2
,,	,,	,,	72	... ...	4·30
,,	,,	,,	74	... ...	4·25
,,	,,	,,	76	... ...	4·25
Control at 90° C. for several minutes				... ...	4·95
Control with enzyme sol. heated to 90° C. for several minutes					1·95

The following experiments show the anti-power of the normal sera of ox and goats for leucoprotease.

Experiment 13.

Powder No. 5. Time 7 days. Ox serum collected two days before and kept on ice.

			Nitrogen as deci-normal acid in 100 c.c. of filtrate
A.	Powder 0·2 gm. + casein sol. 50 c.c. + serum (1 to saline 2) 50 c.c. (heated)	=	36·6
B.	Powder 0·2 gm. + casein sol. 50 c.c. + serum (1 to saline 2) 50 c.c. (fresh)	=	23·8
C.	Powder 0·2 gm. heated + casein sol. 50 c.c. + serum (1 to saline 2) 50 c.c. (heated)	=	6·4

Heated in water bath to dryness from wet state. 100 c.c. tannic acid added. Took 100 c.c. filtrate.

Experiment 14.

Powder No. 5. Time 4 days under sol. 5 c.c. Serum Ox collected previous day. Dil. serum 1, normal saline 3, a total of 50 c.c. of diluted serum in each. The heated serum was raised to 91° C. Substrate, casein sol. 2·5 % in 0·25 % sod. carb. sol.

					Dilute serum				Nitrogen as deci-normal acid in 100 c.c. of filtrate
	Powder		Casein sol.		Heated		Unheated		
(*a*)	0·2 gm.	+	50 c.c.	+	50 c.c.	+	0 c.c.	=	37·0
(*b*)	0·2	+	50	+	40	+	10	=	29·0
(*c*)	0·2	+	50	+	25	+	25	=	22·3
(*d*)	0·2	+	50	+	0	+	50	=	18·0
(*e*)	0·2	+	50	+	50	+	0	=	5·1 control

Tannic acid 100 c.c. 100 c.c. of filtrate taken for estimation.

Experiment 15.

Same conditions with same serum. Time 2 days.

					Dilute serum				Nitrogen as deci-normal acid in 100 c.c. of filtrate
	Powder		Casein sol.		Heated		Unheated		
(*a*)	0·2 gm.	+	50 c.c.	+	50 c.c.	+	0 c.c.	=	26·1
(*b*)	0·2	+	50	+	25	+	25	=	16·3
(*c*)	0·2	+	50	+	0	+	50	=	11·0

Tannic acid 100 c.c. 100 c.c. of filtrate taken for estimation.

Experiment 16.

Powder No. 6. Time 2½ days. Substrate, casein sol. 50 c.c. Goat serum, diluted, serum 1, normal saline 4. Total of 20 c.c. serum in each case.

					Dilute serum				Nitrogen as deci-normal acid in 50 c.c. of filtrate
	Powder		Substrate		Heated		Unheated		
(*a*)	0·2 gm.	+	50 c.c.	+	20 c.c.	+	0 c.c.	=	19·3
(*b*)	0·2	+	50	+	15	+	5	=	17·6
(*c*)	0·2	+	50	+	10	+	10	=	15·2
(*d*)	0·2	+	50	+	5	+	15	=	15·0
(*e*)	0·2	+	50	+	0	+	20	=	13·3
(*f*)	0·2 (heated)	+	50	+	20	+	0	=	2·8

Tannic acid 50 c.c. added. Took 50 c.c. of filtrate.

Experiment 17.

Powder No. 6. Time 2½ days. Substrate, casein 50 c.c. Serum Ox, dil. serum 1, normal saline 4. Total of 20 c.c. dil. serum in each.

					Dilute serum				Nitrogen as deci-normal acid in 50 c.c. of filtrate
	Powder		Substrate		Heated		Unheated		
(*a*)	0·2 gm.	+	50 c.c.	+	20 c.c.	+	0 c.c.	=	19·0
(*b*)	0·2	+	50	+	10	+	10	=	15·5
(*c*)	0·2	+	50	+	0	+	20	=	13·0
(*d*)	0·2 (heated)	+	50	+	20	+	0	=	2·9

Tannic acid 50 c.c. added. 50 c.c. of filtrate taken for estimation.

Experiment 18.

Powder No. 8. Substrate, casein 50 c.c. Time 2½ days. Serum Goat, dil. serum 1, normal saline 4. Total of 20 c.c. dil. serum in each.

					Dilute serum				Nitrogen as deci-normal acid in 50 c.c. of filtrate
	Powder		Substrate		Heated		Unheated		
(*a*)	0·2 gm.	+	50 c.c.	+	20 c.c.	+	0 c.c.	=	21·0
(*b*)	0·2	+	50	+	10	+	10	=	17·4
(*c*)	0·2	+	50	+	0	+	20	=	14·6
(*d*)	0·2	+	50	+	20	+	0	=	2·8

Tannic acid 50 c.c. added. 100 c.c. of filtrate taken for estimation.

Comparative inhibitory effect of Sera from Different Animals.

With regard to the degree of inhibition manifested by normal sera Opie and Barker (1907) find it is not more marked with the serum of the species of animal from which the leucoprotease is taken than with other sera.

Pappenheim's results (quoted by Eisner 1909) do not agree altogether with Opie's. Pappenheim found the serum of the rabbit to be *less* active than that of the dog versus leucoprotease. He however agrees that birds' sera are little, if at all, active.

Delezenne using trypsin as ferment found considerable differences between various animal sera in their anti-power towards this ferment.

Mesnil (1903) working with actinodiastase as ferment found that sera of different species varied in their anti-power towards it and gives the following order of activity: sheep, goat, rabbit, birds.

As the evidence is at present then, normal sera from different animals show varying degrees of activity towards proteolytic ferments. This variability is thought by various observers to be a specific one but as regards leucoprotease at any rate, where two observers have worked on the same animals with the same ferment, they have not infrequently arrived at contradictory results, thus indicating that the differences between sera of individuals of the same species is as great as that between different species.

In my experiments I failed to find any very material difference between the activity of the sera of the various mammals on which I have experimented. In each case controls of maximum digestion, *i.e.* with all serum heated, and also two or more experiments with the fresh serum, one with a larger quantity than the other, were made. This enables one to see that one is not using such a dose of serum that it will give a maximum effect under the given conditions. The amount of serum added in each case is rendered equal by making up to constant amount with heated serum. The following experiments show some of my results:

Experiment 19.

Powder No. 8, 0·2 gm. Time 3½ days. Substrate, 50 c.c. casein sol. Serum, diluted serum 1, water 4. Total amount of dilute serum 20 c.c. in each case.

	Nitrogen as deci-normal acid in 25 c.c. of filtrate			
Dilute serum	Ox	Sheep	Goat	Rabbit
1. With 20 c.c. heated	8·0	7·9	7·65	7·4
2. With 10 c.c. fresh	6·8	6·3	6·15	6·4
3. With 20 c.c. fresh	—	5·25	5·3	5·6
4. Control	1·1	1·0	1·1	1·1

50 c.c. tannic acid sol. added. 25 c.c. of filtrate taken for estimation.

Experiment 20.

5 gm. of powder No. 10; ground with glass in sodium carbonate 0·25 $^0/_0$ sol.; centrifuged; filtered and made up to 500 c.c.

Enzyme, solution as above 20 c.c. Time 2½ days. Substrate, casein solution 50 c.c. Dilute serum, serum 1, water 4. Total of 20 c.c. of dilute serum in each case.

	Nitrogen as deci-normal acid in 50 c.c. of filtrate			
Dilute serum	Ox	Sheep	Rabbit	Pigeon
All serum heated	7·4	7·5	7·3	—
10 c.c. dil. fresh serum	5·55	5·55	4·6	7·55
20 c.c. ,, ,,	4·6	4·4	3·4	—
Control	2·15	2·05	2·3	—

Tannic acid 50 c.c. Filtrate taken 50 c.c.

Experiment 21.

Powder No. 10, ·02 gm. Time 2½ days. Substrate, casein sol. 50 c.c. Serum, 1 serum, 4 water. Total 20 c.c. in each case.

	Nitrogen as deci-normal acid in 50 c.c. of filtrate	
Dilute serum	Ox	Sheep
Serum all heated	16·15	16·9
10 c.c. fresh	15·35	14·65
20 c.c. ,,	13·15	11·8
Control	2·5	2·75

Tannic acid added 50 c.c. Took 50 c.c. of filtrate.

Experiment 22.

Enzyme. Powder No. 5, 0·1 gm. Substrate, gelatin 10 $^0/_0$ 10 c.c. Time 3½ days. Diluent sodium carb. sol. 0·25 $^0/_0$ 10 c.c. Serum (1—6 water), 10 c.c. in each case.

	Nitrogen as deci-normal acid in 50 c.c. of filtrate	
Dilute serum	Ox	Sheep
All serum heated	6·0	5·75
5 unheated	4·70	4·8
10 unheated	4·2	4·3
Control	1·8	1·7

50 c.c. tannic acid sol. added. 50 c.c. of filtrate taken.

Immunity.

Achalme (1901) increased the anti-tryptic power of serum in guinea-pigs by injecting them intra-peritoneally with trypsin. Dean (1901) immunised goats and geese with trypsin but obtained only a trifling increase in anti-tryptic power.

Levene and Stookey (1903) found that the serum of trypsin-immunised rabbits was *markedly* more powerful as anti-body to tryptic digestion. The animals were treated for eight weeks with a dose increasing to 2 c.c. intravenously.

Landsteiner (1907) failed to increase the anti-tryptic power of serum notwithstanding a lengthy immunisation.

Jochmann and Kantorowicz (1908) immunised rabbits with both trypsin and leucoprotease and found an increase of thirtyfold in the power of the serum. The sera of the animals injected with either trypsin or leucoprotease contained an anti-body for both enzymes and saturation of the sera with the one enzyme exhausted it of its neutralising power for both.

Bergmann and Bamberg (1908) doubled the anti-tryptic power of dog's serum by two months' immunisation.

Döblin (1909) failed to increase the anti-power by injecting trypsin into rabbits whereas Meyer (1909) obtained in the same animals an increased anti-tryptic power after the injection of trypsin but not after trypsinogen or kinase.

My own results with leucoprotease are parallel with those of Dean, there being according to the figures (Ex. 23 and 24 below) a slight increase of anti-power. As however the nutrition of the goats was somewhat influenced by the course of injections, *i.e.* loss of weight was noticed, I am inclined to think that such slight variation as is shown in the course of my experiments is as likely if not more likely to be caused by such influences than by any process of true immunisation. Had I used rabbits or dogs the results might have been different.

The goats were treated by subcutaneous injection of preparations of the pus-powder, prepared as mentioned above. This was either given in the form of suspensions in normal saline or sodium carbonate 0·25 °/₀ sol. or by extracts of the powder made by grinding it with glass in saline or sod. carb. sol., centrifuging and filtering. This treatment the animals bore well. The early injections were followed by a good deal of reaction causing firm nodules to appear which were however subsequently absorbed. After the later injections although the dose was considerably increased, this was far less marked. The doses injected are shown in the table as approximate amounts of powder used. I have given the actual ammonia figures of the estimation as well as the percentages of inhibition of the serum.

In estimating this anti-tryptic action the following method was used. 0·2 gm. of the powder was allowed to act in the presence of 2 c.c. and 4 c.c. of the pure serum on 50 c.c. of 2·5 casein solution in the sodium carbonate solution 0·25 °/₀. In each case the serum was

Experiment 23.

Protocols of estimations of anti-action of serum of immunised goats.

	Goat 1 B	Goat 2 B	Goat 1 L
	Dates :—8. 3. '09	9. 3. '09	26. 2. '09
A.	21·0	19·9	19·3
B.	17·4 (20)	16·3 (21)	15·2 (24)
C.	14·6 (35)	13·7 (36)	13·3 (36)
D.	2·8	2·8	2·8
	Dates :—26. 3. '09	26. 3. '09	13. 3. '09
A.	18·8	18·3	19·4
B.	14·55 (26)	15·05 (20)	15·6 (22)
C.	12·0 (40)	13·1 (32)	12·6 (40)
D.	2·2	2·1	—
	Dates :—14. 4. '09	14. 4. '09	26. 3. '09
A.	16·3	15·7	18·8
B.	14·25 (14)	13·5 (16)	—
C.	11·5 (33)	11·4 (31)	12·45 (38)
D.	1·8	1·9	2·2
	Dates :—24. 4. '09	24. 4. '09	—
A.	17·05	—	—
B.	14·5 (17)	14·25 (19)	—
C.	11·9 (34)	12·5 (30)	—
D.	2·3	2·15	—
	Dates :—17. 5. '09	17. 5. '09	—
A.	17·65	17·45	—
B.	14·0 (25)	14·15 (23)	—
C.	12·0 (39)	11·75 (40)	—
D.	3·25	3·1	—
	Dates :—4. 6. '09	4. 6. '09	—
A.	14·85	15·55	—
B.	11·8 (25)	11·85 (28)	—
C.	9·4 (43)	—	—
D.	2·4	2·4	—
	Dates :—15. 6. '09	15. 6. '09	—
A.	18·9	19·6	—
B.	14·4 (28)	14·45 (30)	—
C.	11·4 (46)	11·4 (48)	—
D.	2·65	(2·65)	—

First figures = nitrogen as c.c. N/10 acid in 50 c.c. of filtrate. Figures in brackets = $^0/_0$ reductions. 50 c.c. tannic acid sol. added.

Experiment 24.

Goat 1 L.

Days	Amount of powder injected	Percentage reductions	
		With 2 c.c.	With 4 c.c.
1	—	24	36
11	0·05 a	—	—
12	0·2 a	—	—
13	0·3 b	—	—
16	0·4 b	22	40
29	—	—	38

Goat 1 B.

Days	Amount of powder injected	Percentage reductions	
		With 2 c.c.	With 4 c.c.
1	—	20	35
13	0·2 b	—	—
18	0·2 b	—	—
19	—	26	41
26	0·2 c	—	—
31	0·2 c	—	—
37	0·4 b	14	33
39	0·8 b	—	—
48	—	17	34
71	0·6 d	25	39
80	1·2 d	—	—
89	1·6 e	25	43
100	0·08 IV	28	46

Goat 2 B.

Days	Amount of powder injected	Percentage reductions	
		With 2 c.c.	With 4 c.c.
1	—	21	36
12	0·2 b	—	—
17	0·2 b	—	—
18	—	20	32
25	0·2 c	—	—
30	0·2 c	—	—
36	0·4 b	16	31
38	1·0 b	—	—
47	—	19	30
70	0·6 d	23	40
80	1·2 d	—	—
89	1·6 e	28	—
99	0·1 IV	30	48

a—1 $^{0}/_{0}$ extract in normal saline.
b—suspension in sodium carbonate solution 0·25 $^{0}/_{0}$.
c—suspension 1 $^{0}/_{0}$ in normal saline.
d—1 $^{0}/_{0}$ extract in sodium carbonate solution 0·25 $^{0}/_{0}$.
e – 4 $^{0}/_{0}$ suspension in normal saline.
IV—1 $^{0}/_{0}$ extract in sodium carbonate solution 0·25 $^{0}/_{0}$ given intravenously.

diluted in the proportion of 1 to 4 of normal saline and the total amount of this dilute serum was in each case made up to 20 c.c. by similarly diluted serum previously heated to 90° C. In the preceding table the numbers given in the horizontal columns A, B, C, D represent the following:

A. Maximum digestion with all serum, 20 of dilute, heated.

B. With 2 c.c. serum, 10 c.c. of the dilute serum, fresh.

C. With 4 c.c. of the serum, 20 c.c. of dilute, fresh.

D. Control, enzyme powder heated, serum all heated, 20 c.c. of dilute.

The figures in brackets after B and C in Expt. 23 represent percentage reduction of activity.

Before treatment all three goats gave percentage reduction figures between 20 % and 24 % for 2 c.c. of fresh serum and between 35 % and 36 % for 4 c.c. of fresh serum, while at the end of the course of injections the percentage reduction figures for goats 1 B and 2 B were between 28 % and 30 % for 2 c.c. of fresh serum and between 46 % and 48 % for 4 c.c. of fresh serum.

During the course of estimation it will be noticed that on one occasion the percentage reduction figures fell to between 14 % and 16 % for 2 c.c. of fresh serum and 33 % and 31 % for 4 c.c. of fresh serum. I think it is likely that the 14 % and 16 % are for some reason underestimations but otherwise the 2 c.c. and 4 c.c. estimations run pretty fairly parallel.

In conclusion I wish to express my thanks to Dr Martin, Director of the Institute, and Professor Leathes, Head of the Laboratories for Pathological Chemistry. To Professor Leathes I am indebted for much valuable advice and assistance throughout the progress of the work.

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N° 8

The Problem of Pulmonary Tuberculosis considered from the Standpoint of Disposition.

By WILLIAM BULLOCH, M.D., and MAJOR GREENWOOD, Jun.

IT has long been a commonplace of medical writers that the development of most diseases requires the co-operation of two sets of circumstances. On the one hand, the organism within which the morbid process is to unfold itself, must conform to certain conditions of structure and function. This is the so-called "internal" cause of the disease. On the other hand, some agent, the "external" cause, actually or functionally outside the organism, must exert an effect, peculiar to itself and a property of its own structure, upon the organism which is in process of becoming the seat of the disease. In a large number of instances we now know that the second of the above-mentioned factors belongs to the groups of simply organized plants or animals. Hence the metaphor of "soil and seed" has been conventionally employed to express the belief outlined in the above sentences, and for the present we shall conform to this usage. It is evident that soil and seed, being biologically distinct, may be supposed capable of independent variation. Fixing our attention upon any one kind of seed, we may imagine that in the whole "population" of such seeds all grades of aptitude to germinate may be present. Similarly in the various soils, all grades of fertility for the growth of particular seeds may exist. Since the two variables are independent, the co-existence of any grade of the one with any grade of the other is a function of the relative frequencies in each "population." Evidently if the proportion of very readily germinating seeds in the seed "population" be extremely high, and the proportion of very infertile soils in the corresponding soil "population" very low, then, for that particular seed the choice of soil is relatively unimportant. We see instances of this in such diseases as measles. On the other hand, we have reason to believe that in the case of a disease like leprosy, fertile soils are uncommon. In the case of tuberculous disease, especially in its pulmonary manifestations, medical opinion has undergone fundamental changes. There was a time when the "soil" was regarded as of overwhelming importance, the existence of the "seed" being unknown or misjudged. With the discovery of the tubercle bacillus the trend of popular and, in many places, medical opinion has been to minimize the importance of the soil, if not to disregard it altogether. For reasons too obvious to require statement, it is desirable to know accurately what light has been shed upon this point by the large number of investigators who have studied it. We propose, therefore, to submit the results of a critical inquiry as to the value of work dealing with the respective shares of soil and seed in the genesis of pulmonary tuberculosis. Before, however, entering upon the details of this work it is desirable to refer to certain general aspects of the matter which are of great importance, even if frequently overlooked.

It is to be carefully noticed in the first place that no direct evidence on a matter of this kind can be obtained—so far, at least, as human beings are concerned. Were it possible to choose at random a large number of persons, to divide them at birth into two sets, to bring up one class in contact with exceptional opportunities for infection, and the other in circumstances presenting what one may call usual, normal, or wonted facilities, an examination of the differential incidence of phthisis would settle the question once and for all. No true experiment of this kind has been, or is likely to be, done. Cases often quoted as fulfilling the necessary conditions will not, we believe, stand critical examination. It has, for instance, been suggested that a comparison between the incidence of tuberculosis upon persons in attendance on tuberculous patients and the general rate will lead to results of value. When, however, the subjects of such a comparison are relatives of the diseased persons the question of inherited disposition to tuberculosis is begged. When the subjects of such a comparison are nurses or sick attendants, the difficulties of class incidence are not allowed for. Illogical conclusions of the following nature are frequently met with in popular medical writings. It is pointed out, perhaps, that the incidence of phthisis upon the attendants in some chest hospital or sanatorium for consumptives is much lower than upon the members of the households of tuberculous patients of the working classes. This difference is, then, attributed to the greater care exercised by the attendants in such institutions to avoid infection, as contrasted with the carelessness of hygienic measures and overcrowding which characterize families below the poverty line. Such reasoning is based on the assumption that all persons are equally liable to develop phthisis, given an equality of chances to become infected. In other words, there is a circle in the proof. Precisely the same criticism applies to the inferences often drawn from the higher prevalence of tuberculosis among the poorer classes in general, and in overcrowded dwellings in particular. It will, therefore, be apparent that all arguments of this kind require to be carefully weighed, and that in no circumstance can they be held to be of more than secondary importance. It is equally clear that the increased prevalence of phthisis in the families of diseased persons cannot of itself demonstrate the importance of a disposition to the malady in particular stocks, since such an inference would in turn beg the question of increased opportunities of exposure to infection.

A second preliminary observation relates to the direct transference of laboratory or veterinary experience to the realms of human pathology. Such a transference frequently savours of errors similar to those pointed out in the jests relating to the mechanics of the examination room, where wheels are frictionless and the weights of (very small) elephants may be neglected.

We shall now briefly review some of the more important work dealing with the problem under discussion, outlining first of all the state of medical knowledge prior to the discovery of the infectivity of tuberculosis. Secondly, we shall examine briefly the more recent literature dealing with the immediate problem, and finally some reference will be made to the bearing of these results.

Before, however, dealing with these details, it may be well to consider the concepts, which have been variously described as disposition, "anlage," diathesis, dyscrasia, constitution, habitus, temperament. Unfortunately these terms are used differently by different authors, although at one time they were considered as almost synonymous, and they were the subject of speculation too theoretical to be otherwise than mentioned here. The importance of this aspect of disease was largely developed by the Montpellier school, who separated "diathesis" from "disposition." This is clearly brought out in Sarda's learned study, and even more distinctly in the following definitions from a thesis by Pagnon (1838), from which it appears that a diathesis is an "état général et acquis de l'économie, modification insaisissable dans son essence, pouvant rester indéfinitivement latent et se traduisant à l'extérieur sous l'influence d'une cause quelconque par des phénomènes identiques dans plusieurs points. Il ne faut pas confondre la diathèse avec l'aptitude à contracter les maladies. Cette dernière résulte de conditions particulières de sensibilité qui peuvent se rencontrer sans aucune modification morbifique."

There has also been a growing tendency among the German pathologists to restrict the meanings of such words as "disposition" and "disease-anlage." The recognition of the *causa essentialis* and the *causa occasionalis* of a disease is of importance. Thus, in his excellent introduction in the Krehl-Marchand "Handbuch der allgemeinen Pathologie" (1908), Marchand says: "Ein Krankheitsanlage im eigentlichen Sinne ist eine innere Causa essentialis, die ohne notwendiges Hinzutreten einer anderen Ursache die krankhaften Veränderungen zur Folge hat oder zur Folge haben kann wenn auch in vielen Fällen eine äussere Einwirkung als 'Gelegenheitsursache' (causa occasionalis) die Krankheit selbst hervorruft." After referring to conditions like hæmophilia, colour-blindness, and developmental irregularities, he goes on to say: "In allen diesen Fällen ist die angeborne Anlage eine ihrem Wesen nach uns unverständliche Eigenthümlichkeit der Organisation oder der 'Konstitution' die eine gewisse Minderwertigkeit in dieser oder jener Sphäre bedingt und selbst schon den Keim der Krankheit darstellt.

"Eine andere Reihe von sogenannten Dispositionen oder Anlagen ist *nicht* essentieller Natur. Man versteht darunter die grössere Empfänglichkeit oder geringere Widerstandsfähigkeit des Organismus gegenüber gewissen äusseren Einwirkungen die die eigentliche Krankheitsursache darstellen. Niemals kan eine solche 'Disposition' die Krankheit selbst zur Folge haben."

Birch-Hirschfeld uses the terms "Anlage" and "Disposition" as interchangeable. "Als Constitution bezeichnet man die Gesammtanlage des Korpers, sowohl hinsichtlich der Menge und des Verhältnisses seiner einzelnen Bestandtheile zu einander, als auch nach dem Maass seiner activen Leistung in der einen oder anderen Richtung mit Einschluss seiner Reizbarkeit sowie seiner passiven Widerstandsfähigkeit. Der Ausdruck der Constitution in der äusseren Erscheinung wird als Habitus bezeichnet; während die Art der Reaktion die namentlich auch in den psychischen Bewegungen hervortritt als Temperament benannt wird."

Like Marchand, Baumgarten also distinguishes the "disease-anlage," from the disposition. "Unter Anlagen," he says, "verstehen wir eine derartige in der Zellensubstanz bereits vorhandene Abweichung ihrer

Lebensbewegung von der Norm, welche sich wandelnd durch die in ihr liegende Richtung aus sich selbst heraus früher oder später zu den Erscheinungen führt, oder führen kann, welche zu einem einheitlichen Bilde zusammengefasst wir die Krankheit nennen . . . Unter Disposition verstehen wir den einen der für das Zustandekommen einer bestimmten Krankheit notwendigen Faktoren."

Ribbert refers to the Disposition as "diejenige Eigenschaft eines Organismus welche krankheitserregende Ursachen eine erfolgreiche Einwirkung gestattet."

These quotations will suffice to show the advisability of separating the "disease-anlage" (diathesis) from that condition of susceptibility to disease which is commonly called "disposition." With special reference to tuberculosis this is found running through the whole of medical literature. At first described as the phthisical "constitution" or "habitus," we find, later, such terms as "procatarxis," "predisposition," or "diathesis" as essential elements in the conception of this disease. In point of time we find in the classical writers, mainly the idea of constitution or habitus. Later, writers like Richard Morton, Desault, and others, described accurately what we should call the "predisposing" factors; while, at a still later period, Bayle founded the doctrine of the tuberculous "diathesis" in the strict sense of the word. Subsequent to the period of Laennec and Louis there was a gradual return to the doctrine of disposition which, in combination with the *causa essentialis*, determined the occurrence of a tuberculous infection. The relative importance of the disposing as contrasted with the infective element is the subject under discussion.

The description of the consumptive type created by Hippocrates runs like a thread through the whole fabric of medical literature. In the third book of the "Epidemics" he says: "The form of the body peculiarly subject to phthisical complaints was the smooth, the whitish, that resembling the lentil, the reddish, the blue-eyed, the leucophlegmatic, and that with scapulæ having the appearance of wings."

In the pseudo-Hippocratic treatise "Prorrhetics" it is stated that phthisis is difficult to remove if hereditary.[1] In the sixth book of the "Epidemics," which is probably spurious, there is a passage which suggests a belief in the constitutional factor. In Van der Linden's Latin translation it is given: "Morbus tabidam speciem inducit si talis ex natura aeger fuerit ex tali morbo superstes evadet; et alia eodem modo."[2]

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[1] Adams's translation, i, p. 259.

[2] Leiden, 1665, i, p. 817.

Galen, in his account of the phthisical type makes the same remark as Hippocrates with respect to the scapulæ, and he notices the narrow, shallow chest which frequently figures in later accounts.[1] Celsus, while reproducing Hippocrates's clinical description, does not refer to the existence of a disposition, but he recommends taking "a long voyage, change of climate, taking care to remove to a grosser air" (Book III, chapter xxii).

Pliny (23—79 A.D.) also makes no reference to disposition, but incidentally we may remark that he refers to a treatment popularly supposed to be quite modern: "Silvas eas dumtaxat quae picis resinaeque gratia radantur utilissimas esse, phthisicis, aut qui longa aegritudine non recolligant vires satis constat; et illum coeli aera, plus ita quam navigationem Aegyptiam proficere plus quam lactis herbidos per montium aestiva potus."[2]

The post-Galenical writers, Aretaios, Rhuphus of Ephesus, Oreibasios Aëtios, Alexandros of Tralles, and Paulus of Aegina were collected in a single volume by Henry Stephans in 1564. Unless the phrase of Aëtios (de febribus hecticis ac tabidis). "Eas vero quae in tabem procedunt, sanare impossibile est. Natura enim ipsarum calida est et sicca,"[3] may be considered to have reference to a constitutional condition, we have found no new facts pointing to this belief in their writings. Avicenna (eleventh century), in the third book of the Canon, deals with the point at some length. His account is transcribed in a footnote,[4] and appears to be little more than an amplification of the Hippocratic and Galenical tradition.

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[1] *See* Adams's translation of Hippocrates, p. 353, note 3.

[2] Book xxiv, cap. 6.

[3] "Tetrabiblon," II, i, cap. 92.

[4] Avicenna, Lib. III, Tract 4, cap. 20 (Alpagus's translation, edition 1557, p. 271): "Isti sunt alati habentes pectus strictum, habentes spatulas nudas carne et proprie posterius habentes spatulas declives ad anteriora eminentes et quasi unicuique eorum sunt duae alae et quasi spatulae ejus sunt abscissae ab adjutorio et ante et retro; et habentes colla longa et inclinantes ei ad anteriora. Et quandoque praecedunt guttura ipsorum et eminent. Et in pectoribus quidem ipsorum, quae sequntur ea, multiplicantur ventositates et inflatio est in eis propter parvitatem pectorum eorum. Si autem cum hoc est debilitas cerebrorum recipientum superfluitates et cum hoc non digeruntur cibi, tunc jam complentur conditiones: et proprie si fuerint humores eorum acuti cholerici. Et formae quidem susceptibiles phthisis velociter cum alatione prædicta, sunt lenes absque pilis albae declinantes ad russedinem; et etiam corpora dura spissa propter illud quod accidit eis de ruptione venarum. Et complexio susceptibilis illius, est ejus cujus complexio est frigidior. Et aetas in qua multiplicatur phthisis est illa quae est inter octavum et decimum annum usque ad adventum (XXX) annorum. [N.B., the Hippocratic limits are 18—35 years.—W. B., M. G.] Et ipsa in regione frigida, est plus propter illud, quod accidit in ea de ruptione venarum et sputo sanguinis plurimo. Et tempus in quo multiplicatur illud, est autumnus."

From these varied quotations it will be seen that while a belief in the existence of a physical type or habitus specially prone to phthisis is to be found in the great medical classics it is certainly not clear that very great stress was laid on the matter. As we shall show, it is clear, however, that the idea that phthisis is an infective disease was not so entirely neglected by the ancients as has often been thought.

As a considerable proportion of the medical writings of the fifteenth and sixteenth centuries are purely derivative, we need not stay to analyse them. Coming to the seventeenth century, we may point out that Christopher Bennet, the first English writer on phthisis (1654), refers to the hereditary nature of the disease, which by the time we reach the eighteenth century had become a mere commonplace of the literature. This had also been emphasized by Lommius (1560) more than a hundred years before. While expatiating on the fact that phthisis is chronic and infallibly fatal, and incident to slender persons with narrow chests, long necks, prominent scapulæ, distorted legs, and to those persons subject to frequent defluxions, he adds, "idem malum magna ex parte a parentibus ad liberos transit; sic ut integras etiam familias veluti jure quodam hereditario oppugnet."

With the appearance of Richard Morton's "Phthisiologia" (1694) we have emphasis laid on what we should call the disposing causes, in addition to the actual constitutional factor in the disease. He says (p. 64): "The cause of a consumption of the lungs in general is a vitiated disposition of the mass of blood of the spirits in the nerves, contracted gradually from several procatartick or predisposing causes in which the sharp or malignant serum or water of the blood being separated by the soft or glandulous substance of the lungs does stuff, inflame, and at length also exulcerate the lungs themselves, which is the immediate cause of this distemper." The "procatartick" causes specially referred to are: (1) Stopping of usual and necessary evacuations, monthly courses, child-bed purgations, old sores, fistulas, issues, sweating in the soles of the feet; (2) passions of the mind—fear, grief, anger; (3) intemperance in eating and drinking; (4) want of exercise; (5) night studies and long watchings; (6) sleeping in the day, and sleeping much; (7) an ill air; (8) "an hereditary disposition from the parents does very often bring a consumption of the lungs when everybody knows very well that those who come of consumptive parents are apt to fall into the same distemper"; (9) an ill-formation of the breast, whether it be natural or accidental. "I call that natural when the breast is narrow, the neck long, the shoulder-blades stand out like wings, and I call accidental when there is crookedness or distortion of the breast, whereby not only the parts of the breast, and especially the lungs, being once weakened in their tone, are rendered subject to the flux of rheumes." (10) Infection; (11) chalky stones and such like in the lungs; (12) some preceding distemper. "The body being thus predisposed to a consumption by these and other such like procatartick causes, the more immediate cause of this distemper is for the most part the taking of *cold*, from whence it comes to pass that in a body disposed in such a manner by a load of humours or water continually derived from the distempered habit of the body into the lungs a cough is caused that is not easily shaken off."

These views of Morton were much in advance of his time, and slightly paraphrased, may be looked upon as the opinions largely held at the present day. They were accepted in the main as current teaching shortly after his time. The popular medical view is seen in the following passage from a not very profound observer, Salmon. In his "Practice of Physick" (1716) he says: "To these previous symptoms you may add an hereditary disposition which gives more certainty to all the former signs. For as usually one troubled with gout or stone, asthma, king's evil, mania, frenzy or epilepsy usually beget children affected with the same diseases, so likewise one troubled with a phthisis or a consumption may beget a child apt to have the same disease." Referring to the symptoms of phthisis, he adds that, "though authors and even daily experience have approved all these signs to be the characteristics of a confirmed phthisis, yet it is as true that they do not all happen to every patient, but some to one patient, and others of them to another, and that according as their strength, nature, constitution, and habit of the body will admit them."

It was during the first third of the eighteenth century that Pierre Desault (1675-1737), a distinguished physician of Bordeaux, published his important views on the relationship of tubercles to the production of phthisis. Basing his views on long-continued pathological anatomical observations, he was the first to assert that the causes of phthisis were "conjoint" and "antecedent," the conjoint cause being that "dont la présence fait la maladie et la destruction la guérit. Cette cause conjointe de la phthisie nous l'établissons dans des tubercules et concrétions qui se sont formez dans le substance du poulmon et qui sont répandus dans ses lobes. Ces concrétions ressemblent à des grains de grêle et sont de différentes grandeurs." Desault distinguished three types of persons becoming tuberculous from antecedent causes, viz.:

"(1) Ceux qui ont la poitrine serrée, le col long, les épaules relevées comme des aïles d'oiseau y sont les plus sujets; (2) ceux qui ont le malheur d'être nez de parens phthisiques y sont très-enclins, car cette maladie à cela de commun avec bien d'autres, qu'elle se traduit par héritage; (3) ceux qui sont engagez à vivre avec les phthisiques à les servir, peuvent prendre le mal, pour peu qu'ils y ayent de disposition; car il est contagieux, comme nous le serons voir dans la suite; ainsi les femmes qui servent leurs maris phthisiques le prennent souvent d'eux et les maris de leurs femmes."

The famous van Swieten, in his commentary on Boerhaave, apropos of the latter's aphorism regarding the gravity of hereditary phthisis gives the details of a family observed by himself. The mother came of a phthisical stock, and died of phthisis, together with the rest of her brothers and sisters, before the age of 30. The husband, a healthy man, lived to be over 80. Of the four children of the marriage which survived infancy three died of phthisis, while the fourth, "reliquorum sorte territus, tempestivis venae sectionibus funestam haemoptien cavit," a plan of operations which he seems to have carried out with such vigour as eventually to die not of phthisis but of its prophylaxis. The children of this man were healthy at the time of record.

These excerpts will show the current teaching on the nature and causes of phthisis towards the last third of the eighteenth century. But a new era was setting in. The foundations of the morbid anatomy of phthisis laid by Desault were sunk deeper by William Stark and Matthew Baillie in this country, and the recognition of the macroscopic "tubercle" as a constant factor in consumption was almost bound to alter the current of thought on the ætiology of the disease. This alteration came about particularly through the observations of G. L. Bayle, to whom we largely owe the idea of a tuberculous *diathesis*, an idea destined to play a dominant rôle in all discussions on this subject, down to the researches of Villemin and Koch. The evolution of the idea of a diathesis as opposed to mere disposition is to be found in Bayle's paper, "Remarques sur les tubercules" (1803), and was based on the observation of tubercle, not only in the lungs but in many other parts of the body, showing *apparently* that the condition is not only local but general. As this doctrine was new, and was destined to be the cause of heated conflict during the subsequent sixty years of medical research, we quote Bayle's exact words. On p. 28 of the above-mentioned memoir, under the title, "Considérations pathologiques relatives aux tubercules," he says: "Il n'est pas rare de rencontrer

chez le même sujet des tubercules très-nombreux dans divers organes; par exemple, dans les poumons et le mésentère, dans le mésentère et le foie; dans les poumons, les reins et la prostate; ou même dans la plupart des organes où nous les avons précedemment décrits. Cette simultanéité paraît indiquer que la nature de tous les tubercules est identique, et elle montre qu'il existe souvent dans l'économie une disposition particulière qui détermine leur formation. Peut-être conviendrait-il de désigner sous le nom de diathèse tuberculeuse, tendance à la production des tubercules."

The opening years of last century witnessed extraordinary developments with regard to the whole question of phthisis and the establishment of the doctrine of tubercle as the morbid substratum of diseases which had hitherto been classified from their semeiological resemblances only. This development we owe largely to the magnificent work of Laennec. He laid the basis of the doctrine of tubercle as a definite pathological change irrespective of its site; he distinguished the nodular and diffuse forms of the infiltration, and established the concept of a "tuberculous degeneration," or "tuberculous affection," which was the real basis of conditions which had hitherto been misunderstood. The whole problem was elaborated by Laennec into the comprehensive concept of "tuberculosis," the real essence of which was some change taking place within the body as the result of various predisposing factors, many of which he specified. A great many of the details of Laennec's teaching, especially as regards the evolution of tubercle, were the subject of heated discussion immediately after his publications. To this period belong the researches of Gendrin, Lobstein, Andral, and particularly Louis in France; Barron, Carswell, and James Clark in England; Schönlein and Canstatt in Germany; and Rokitansky in Austria. But the dominating and accepted view was that tubercle as a general pathological process is something inherent to the body and dependent on a diathesis, or constitution, which is lighted into activity by various predisposing causes. Thus Andral says that "there are certain habits of body, certain constitutions, innate or acquired, that predispose to the development of tubercle, that is to say, it most frequently appears in those constitutions, and it is a remarkable fact that it is in such cases especially that we observe the morbid production developing itself in several organs at once, both in the young and in the old" (p. 537). Further on he says: "I think I am borne out by facts in the two following propositions—(1) that the tubercular diathesis is in indirect proportion to the development of the constitution above described;

(2) that in proportion as this constitution becomes less strongly marked the tubercles also become less frequent and especially occur in fewer organs at once." The peculiar constitutions he refers to are met with in individuals whose skin is very fair, and in whom the prominences of the cheek exhibit a red tint as if it were laid on with a brush, which forms a remarkable contrast with the dead white of the rest of the cheek. Their muscles are soft and slender and show little contractile power, their blood is serous and deficient in fibrin and colouring matter, and among their secretions the mucous predominate. "It seems to me," he says, "that such persons preserve in adult age several of the characters belonging to the age of childhood, considered in its healthy or morbid state; their organization is in a manner arrested in its development."

That the constitution could be transmitted was held as a fixed belief. It was also held, however, that the disposition might remain latent. Thus the illustrious Thomas Young wrote in 1815: "There is a very general prejudice respecting the transmission of a tendency to scrofula which is often the source of much unnecessary uneasiness; it is supposed by many to constitute a marked and decided character, incapable of being increased or diminished, and which must in all probability produce at some time or other the most melancholy effects in the individual and in his descendants. In fact, however, no man was ever born incapable of becoming scrofulous, and in this sense every person may be said to possess more or less of a scrofulous taint, which may become mischievous or fatal to all, under improper management, but which in other circumstances may easily remain latent throughout life." We commend the calm good sense of this passage to those enthusiasts who teach that "soil" is everything, just as we advise the more numerous band of zealots who hold seed to be all important to peruse another passage from the same work, which we shall cite directly.

The diathetic doctrine of Bayle, which spread through the works of the early years of the nineteenth century, appeared on so firm a basis that even with the rise of exact morbid anatomy it still continued as a fundamental belief. Thus in Rokitansky we find the prevailing idea of a dyscrasia: "Das Exudat kann in einzelnen Fällen das lokale Product einer Entzündungsstase sein, gemeinhin ist diese aber selbst die Lokalisation der Tuberkelkrasis, und das tuberculöse Exudat somit grösstentheils das Product allgemeiner dyskrasischer Vorgänge." As manifestations of this dyscrasia, Rokitansky, on the basis of his enormous pathological experience, formulated his conception of the "phthisical habitus," viz., a heart too small for voluminous lungs in a long narrow thorax, a certain crasis of the blood favouring exudations, a delicate arterial wall, a weak muscular system, &c. In spite of the additional powers given to Virchow through the perfection of pathological histology, even he was dominated by and permeated with the idea of a tuberculous disposition or dyscrasia. In the second volume of his great work, "Die krankhafte Geschwülste," published so late as 1864, he still maintained that the cause of tubercle is to be sought in a general or local disposition leading to a vulnerability of the tissues. "Die Tuberculose," he says, "ist wesentlich eine Krankheit des extra-uterinen Lebens und wenn sie hereditär sind, was nicht bezweifelt werden kann, so ist sie doch nicht congenital. Hereditär ist sie nicht als Krankheit sondern als Disposition. . . . Die Gewebe sind die Träger der Disposition, der hereditären Vulnerabilität."

It was shortly prior to the publication of Virchow's great work that Buhl in Munich had introduced into the whole subject a new conception in so far as he emphasized the constant association of miliary tuberculosis with a pre-existing caseous focus, and he correctly interpreted these two factors as those of effect and cause. According to him tubercle is a specific resorptive disease originating in the absorption of a special poison into the blood, and he considered that the inheritance of the disease consisted in the transference of the special poison to the germ cells. In 1865 came the epoch-making communication of Villemin, in which he was able to assert that tubercle is a specific infective disease with its cause residing in an inoculable agent. His subsequent observations were collected in his great work, "Études sur la tuberculose," published in 1868. In spite of his demonstration that tuberculosis is due to a specific inoculable virus, he was not carried away with the idea that the mere presence of this virus explains all the phenomena of the disease, for he particularly emphasizes the fact that in the prophylaxis of tuberculosis two points are of cardinal importance, viz., the knowledge of the existence and condensation of the morbid ferment, and "l'aptitude du milieu organique." "L'hygiéniste," he says, in conclusion, "ne doit pas perdre de vue les principes qui reposent sur ces deux ordres de faits." In his last important paper, "De la propagation de la phthisie" (1869), he sums up in masterly style his views on the dissemination of the disease, views which it may be said have originated the whole trend of medical opinion since his time. By series of experiments he demonstrated anew the infectivity of phthisical sputum by inoculation, inhalation, or ingestion; pointing out the danger of sputum getting into the air as dust. He recognized the fact that disease than it is of conveying glanders. The virulent principles of both diseases do not seem volatile enough to be conveyed by air. Phthisis does not seem to spread among the attendants in consumptive hospitals in London because the expectorated matters are received into places where they can decompose. The most favourable conditions for contagion are to be found in the dwellings of the poor and in common habitations, such as barracks, convents, prisons, &c. He concludes that the prophylaxis of phthisis may be summed up by avoiding the morbific agent and augmenting the resistance of the organism. In reading the work of Villemin one cannot but be struck by the temperate view he takes even in the presence of the momentous discovery he had made that tubercle is an infective disease, for by demonstrating that its cause is essentially external he had destroyed for ever the belief that tubercle owes its origin to some internal transformation of the tissues excited by an accidental or occasional cause. Even in the light of the discovery of a specific virus he realized the importance of the terrain or soil on which this virus is to flourish.

The fifteen years following Villemin's publication may be described as the "Sturm und Drang" period in the research on tubercle. Its culminating point was Koch's famous paper, "Ueber Tuberculose," delivered before the Berlin Physiological Society on March 24, 1882.[1] In it we have the isolation of the actual virus of tuberculosis and the demonstration of the unity of tubercle as a pathological process—a complete and irrefutable confirmation of the doctrines of Laennec and Villemin. In his completed paper ("Die Aetiologie der Tuberculose," 1884) Koch also refers to the hereditary predisposition to tuberculosis in a particularly temperate manner. "Against the view," he says, "of the unity of all forms of disease caused by tubercle bacilli is the material difference in the course taken by the disease in different individuals of the same race, and in their susceptibility to tuberculous infection. These are, however, phenomena which appear in a more or less marked manner with all infective diseases. It is usual to get out of the difficulty by supposing that there is a difference in the predisposition to the disease both as regards taking it in the first place and the greater or less severity of the course it runs, but this specifies the fact rather than explains it. Some of the dissimilarities in the pictures presented by cases of tuberculosis may be ascribed to differences in the seat of infection. Then, too, the quantity of infective material coming into operation in the first place seems to be of essential importance. . . . .

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[1] *Arch. f. Anat. u. Physiol.* (Physiol. Abth.), Leipz., 1882, p. 190.

But although a good many of the phenomena classed together under the head of 'predisposition' may be referred to simple and easily explained conditions, some facts still remain difficult or impossible to interpret, compelling us for the present to accept the view of a varying liability. Most important of all is the striking difference between the course of tuberculosis in children and in adults, and again the undeniable predisposition to tuberculosis which exists in some families. In the latter instance many of the cases of illness, where predisposition is supposed to be an important factor, might rather be ascribed to increased opportunities of infection, and there are also peculiar predisposing influences connected with the family constitution to be thought of, such as a tendency to catarrhal affections of the respiratory organs and imperfect development of the thorax. Still many carefully observed cases remain which cannot be explained in this manner." Koch also emphasized the fact that the disposition to the disease must be variable as regards time, for a tubercle might attain a considerable size and then contract and cicatrize. Such temporal changes show that there is nothing against the view that conditions favourable or unfavourable to the tubercle bacilli are present in some human beings not only temporarily but for the whole lifetime. With regard to the question of inheritance in tuberculosis, he expressed himself quite definitely in the statement that it "is explained most naturally by supposing that the infective germ itself is not inherited but rather certain peculiarities favourable to the development of germs which may later on come into contact with the body—in fact, it is the predisposition to tuberculosis which is inherited."

In spite of the temperate views expressed both by Villemin and Koch, the founders of the doctrine of infectivity of tuberculosis, many of their successors at once adopted much more advanced views, in which an overwhelming importance was ascribed to the infective agent. We have, however, seen the belief in a special susceptibility of persons conforming to a particular physical type recurring at intervals from the time of Hippocrates to the end of the nineteenth century. Great and often untrustworthy as may be the influence of dogma and tradition, it is surely unscientific to pretend that this belief is without any other warrant than mere authority. Evidently, if it is once admitted that a phthisical type exists, then it is clear that the question of an inherited disposition becomes of more than academic interest, since few will deny that the physical characters of mankind are heritable in a high degree. In our opinion there is no warrant for calmly ignoring, as has been done,

CONTAGION.

It may be a fact that in pre-bacteriological days too little stress was laid upon the communicability of phthisis, but it is a vulgar error to suppose that the matter was never discussed. Aristotle, in the "Problems,"[1] inquires why consumption, but not dropsy, can be communicated to persons near at hand. "A consumption infects because it renders the breath bad and heavy. And those diseases are caught the most rapidly of all others which arise from putrid breath—such, for instance, as are pestilential diseases. But he who approaches such disorders as these draws to himself a breath of this kind. He becomes diseased, therefore, because such breath is noxious. But he becomes diseased through one person because this one person alone has a putrid breath and others have a different breath. And he is infected with the same disease because the breath of a consumptive person is vitiated through the consumption alone." Francis Adams, the learned translator of Hippocrates, adduces reasons for holding that Isocrates thought consumption to be contagious. Galen says explicitly that "it is dangerous to pass the whole day with the consumptive, and in general with all those persons whose diseases generate putrid effusions, so as to make the house in which they live offensive." Avicenna also considered consumption to be contagious.

Coming to modern times, Lommius (1560) has the following striking passage: "Jam etiam sputum mistum purulentum, idemque fœtidum quod halitus etiam contagione imprudentes alios labefactat, assiduaque febris, quae opportuna cibo tempora eripit et multam sitim movet, in tenui corpore certum esse periculum ostendunt." Morton (1694) was convinced that consumption is propagated by infection: "For this distemper (as I have observed by frequent experience), like a contagious fever, does infect those that lye with the sick person with a certain taint." He refers to the case of a phthisical youth who appeared to have infected both his sister and a nurse with the disease. We have already quoted similar views as expressed by Desault. The question was also discussed by Frederick Hoffmann (1740), who cites other observations by Riverius and Schenck. In his twenty-second letter, treating of purulent and ill-conditioned empyema and phthisis, Morgagni (1765) says that Valsalva, when a young man, having been in danger of a consumption, as is written in his life, was rather cautious of dissecting bodies that had been wasted away by disorders of this kind, and Morgagni adds: "Ego vero ut me tibi aperiam illa fugi de industria adolescens et fugio vel senex, tunc ut mihi nunc ut studiosae, quae me circumstat juventuti prospiciam, cautius fortasse quam opus sit; at tutius. Itaque non multa ille, ego vix aliquod dissecui." Van Swieten, in his commentaries on Boerhaave's aphorisms, refers to a youth who spit up such fœtid matter in the morning that he (Van Swieten) (who was "not so nice as to be easily affected with this kind of thing") was scarce able to endure the stench. "Certe juvenis ille cujusmodo mentionem feci, infecit sororem et ancillam quae ipsae in morbo assidue ministraverant." The value of Van Swieten's testimony is perhaps discounted by the somewhat improbable story he relates, according to which a man's wife expiring of a consumption, giving him a farewell kiss, all that part of his chin which her lips had touched remained ever after smooth, though the beard grew thick all round ("postea ille locus glaber mansit, licet densa barba cresceret in omni ambitu"). Cullen (1796), while not denying the possibility of contagion, had never, among many hundreds of instances of the disease, seen one which to him could appear to have arisen from contagion. Heberden (1803), however, a contemporary of Cullen, had seen "several die of consumption in whom infection seemed to be the most probable origin of their illness, from their having been constant companions or bedfellows of consumptive persons."

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[1] Taylor's translation, vii, p. 8.

In Italy in particular the contagious origin of phthisis was widely but not universally accepted. Thomas Young, for example, cites the writings of Borsieri (1782), who remarked that the College of Physicians of Tuscany had decided against the contagious theory, but had not succeeded in convincing the authorities of the uselessness of precautions. Boiseau, in his learned paper, also cites Cocchi (1761) as an anti-contagionist. Young's own views on the subject appear to us worthy of reproduction in full. He says:—

> "It has been much disputed whether or no consumption is ever capable of being communicated by contagion, and it must be allowed that it would be extremely difficult to produce any strong demonstrative evidence of the fact. It may often have been observed that the husband or wife of a consumptive person has died consumptive within a few months after that person's death; but it will appear upon calculation, that this circumstance must happen in London more than once in about two months, before we can deduce from it any proof of the probable connexion of the two events. For one, at least, of 50 adults may be expected to die annually, and consequently one of 200 by consumption, or one out of 400 in six months; and if we estimate the number of married persons dying annually of consumption at 2,000, we may expect to find five instances of apparent contagion without any real foundation. The evidence which has been adduced to prove the infectious nature of consumption has

been by no means of this precise nature, but it has consisted in great measure of absurd and almost superstitious accounts of the propagation of the disease by articles of dress and furniture. At the same time, there has been a very general impression among medical men of the best judgment, with the best opportunities for observation, that they have met with unequivocal instances of the communication of the disease, especially between husbands and wives, and in families partaking of the same hereditary constitution; and Dr. Rush has given an account of a consumption manifestly contagious, which spread from the family of the proprietors of an estate among the negroes, who were neither related to the first victims, nor had been subjected to fatigue or anxiety on their account. I have myself known an instance of a carpenter who died of a laryngeal consumption, and whose wife died soon after, as I was informed, of a disease precisely similar, and I should think it unjustifiable, from a full confidence in the absolute impossibility of infection, unnecessarily to expose any person who appeared to have the slightest predisposition to the disease to any intimate communication with a consumptive patient—as, for instance, to sleeping in the same bed or living constantly in the same room, which is nearly the extent of the apprehension expressed by Galen himself, who observes that it is dangerous to pass the whole day with consumptive persons." (Young, pp. 46, 48.)

Had this passage been written in 1910 instead of 1815, we are not acquainted with any evidence which would disentitle it to be regarded as eminently worthy of the author's scientific reputation.

Laennec, while referring to the fact that consumption had long passed for a contagious disease "by the people, by magistrates, and some medical men, especially in the southern parts of Europe," did not think that it appeared to be contagious in France. "We frequently observe," he continues, "among the poorer classes, a numerous family sleeping in the same apartment with a consumptive patient, and a husband occupying to the last the same bed with his wife without any communication of the disease. The woollen apparel and the beds of consumptives which it is the custom to burn in certain countries are not generally washed, and yet I have never seen the disease communicated by them. It would be well, nevertheless, were it merely on the score of prudence and cleanliness that greater precautions were taken in this respect. It is well ascertained that a disease not usually contagious may become so in certain circumstances." Forbes, whose translation of Laennec we have just quoted, adds in a footnote that the opinion of the great majority of medical men in this country is opposed to contagion, an opinion which he considers is justified equally by statistical facts, by the truths of pathology, and by analogical reasoning. Forbes himself had never witnessed among the thousands of cases he had attended one unequivocal instance of transmission by infection. Sir James Clark, one of the most careful observers who have written on phthisis, was of the same opinion. That the tuberculous constitution was transmitted, however, he considered to be one of the best established points in the ætiology of the disease. Louis, likewise, whose work is a mine in which most subsequent writers have quarried, was no supporter of the doctrine of contagion. Sir Thomas Watson, in his well-known treatise, discusses the question of the contagiosity of phthisis in his usual temperate manner, and concludes that the "disorder does not spread by contagion."

We have referred above to the epoch-making work of Villemin, who established beyond doubt the existence of contagion as a factor in tuberculosis. About the same time William Budd arrived at a similar conclusion by a different process of analysis. He noted the importance of the geographical distribution of consumption in past and present times, and especially its great fatality now in countries which when first discovered by Europeans were entirely free from it. He also drew attention to the prevalence of tuberculosis in convents, harems, barracks, and penitentiaries, where the inmates live in relatively close contact with each other. It may be pointed out, however, that the exact data on which Budd based his assertions are not given in detail in his paper. Shortly after the publication of the writings of Budd and Villemin reports began to appear which seemed to favour more and more the idea that phthisis is communicable from one person to another (Bergeret, Wernich). It was not that these observations were of a kind new to medical literature, but that the interpretation of the findings was different. Immediately after Koch's discovery the question created enormous interest. It was one of the principal themes for discussion by Rühle and Lichtheim at the second Congress for Internal Medicine in Wiesbaden in 1883. Both these authors considered that, although contagion was not doubted, the question of a disposition was of the greatest importance.

One of the most ardent supporters of the contagion doctrine was Cohnheim, and it is probably largely through his dominant influence that the idea of a disposition came to be suddenly discredited. "The assumption," he says, "of an inherited predisposition to this disease is not, in my opinion, necessary to explain its subsequent development in the children of tuberculous parents. For the fact that several members of a family become tuberculous proves nothing but the presence in it of conditions calculated to give rise to tuberculosis; and what condition could be more favourable to its production than the presence of a phthisical patient in the family? When a child nourished by its tuberculous mother becomes tuberculous it is less far-fetched to assume the communication of the disease by the mother's milk into which the virus may pass than the inheritance of a diseased constitution. . . . In short, where the opportunities for acquiring tuberculosis are so manifold we must be furnished with proofs of at least a different character to those already brought forward before we can accept as demonstrated the existence of an hereditary predisposition to the disease" (p. 735). As late as 1899 a similar extreme position was taken up by Cornet in his exhaustive work in "Nothnagel's specielle Pathologie und Therapie." He dismisses the question of hereditary disposition in a few pages, finishing up with the following conclusion, printed in extra heavy type: "Ein maassgebender Einfluss auf die Entwickelung und Verbreitung der Tuberculose kommt auch der hereditären Disposition nicht zu." Apart from a hereditary disposition he considers that *dis*position is often really *ex*position, and that is the main line of his argument.

It may be pointed out, however, that in the period immediately following Koch's discovery great activity was manifested in civilized countries to settle the question of the contagiosity of phthisis. Collective investigations, so-called, were instituted—e.g., in America, Germany, France, Italy, and England. The results of the opinions of some 50,000 doctors who participated in this gigantic undertaking have been described by Cornet as "mehr als bescheiden." We may particularly refer to the "Collective Investigation Record, edited for the Collective Investigation Committee of the British Medical Association by Professor Humphry and F. A. Mahomed," (1883). In spite of the fact that the Association was urged to action by preliminary exhortations of coryphæi like Sir William Gull and Sir James Paget, the net result of the endeavours of British physicians was indeed modest. Out of many thousands who were circularized 1,078 made returns. A great many (673) of these returns were simply negatives containing the word "no," meaning that no case of contagion had been observed by the member making the return. These returns, admitting of no further analysis, were set aside. The remaining 405 observers were classified as follows, viz.: (1) 261 with affirmative answers, (2) 39 doubtful, and (3) 105 negative. In Class 1 there were several groups, as follows:—

(A) Return of cases observed between husband and wife only	158
(B) Return of cases observed between members of the same family: between parents and children, brothers and sisters, as well as between husband and wife	81
(C) Return of cases observed between persons unrelated as well as between persons of the same family	13
(D) Returns containing cases between unrelated persons only	8

A study of the details of many of the returns shows that they cannot withstand criticism. Thus one of the eight cases alleged to have occurred between unrelated persons was the following: Observation 260.—"A female, aged 24, mother and father both alive and healthy, *but all the maternal aunts died of phthisis* (*!*) Before her confinement she had slept constantly with a person, no relation, who had phthisis. She was before this free from all symptoms of the disease."

Among the negative returns we may direct special attention to Observation 392, which the key informs us was that of Sir Clifford Allbutt. It runs: "Although my attention has been given for many years to this question, I never have observed a case of even probable transmission of phthisis from one person to another."

Observation 311 was that of Sir William Church: "Like everyone else I have seen cases of two, three, or more of a family, generally sisters, suffering one after the other, but that does not enable me to answer the question in the affirmative."

The general result of collective investigation records in this country and elsewhere would seem to show that the value of the contagion factor in phthisis is not so easily determined as many have supposed.

We may conclude this section with some reference to the views of August Hirsch (1886), than whom no one possessed a wider range of knowledge or could draw his deductions in a more logical manner. He discussed the bearing of the infective theory of the origin of phthisis upon actual facts that had been ascertained by historico-geographical and statistical research, and concluded that "the contagious transmission of phthisis plays but a subordinate part in the spread of the malady, and it will be all the harder to exclude that conviction if we bear in mind that many parts of the world which offer in respect to sanitation favourable conditions for the occurrence of the disease have little or none of it, although there has been no lack of imported cases. All this seems to show that the transmission from person to person takes place only under certain conditions, and I hold it to be at least doubtful whether these conditions are created simply by individuals living together in close intercourse. . . . But no one need hesitate to affirm that the decisive factor in the genesis of the disease has to be looked for in the *disposition* of the individual to fall into the same." Our quotation from Hirsch brings us to a period now a quarter of a century remote, and it will be our endeavour in the next section to determine what advances have been made in this period, and what is the trend of opinion to-day on the doctrine of Disposition as a factor in phthisis.

The chief branches of inquiry through which it has been sought to advance the problems of infection and disposition to pulmonary tuberculosis have been (1) bacteriological and pathologico-anatomical; (2) epidemiological and statistical; (3) clinical.

With regard to the first of these a large amount of evidence has accumulated to show that the susceptibility of animals to a particular infective agent varies with the animal, and even among species of the same animal. The gradual deepening of the whole problem of immunity compels one to assume that the disposition is a variable factor, and in a large number of instances the study of acquired immunity has served to bridge the chasm between disposition and infection. In the case of tuberculosis in particular, however, unexpected difficulties have been encountered, in so far that it is not at all clear in what manner immunity is produced against the tubercle bacillus.

The pathological anatomists have made numerous contributions of first-rate importance. Among them is the establishment of the fact that tuberculous lesions are found in an enormous number of the autopsies made on adults. The very careful series of cases studied by Naegeli, and in particular by Burkhardt, show that over 90 per cent. of adults have tuberculous lesions. If the tubercle bacillus were ubiquitous, and we could assume that the opportunity of infection were equal for everyone, the occurrence of such an enormous amount of tubercle, compared with the fact that only, say, one-seventh of the human race die of tubercle, would show that the remaining six-sevenths possessed a degree of immunity which enabled them to overcome an infection which had already taken place. The problem is, however, by no means so simple, inasmuch as it remains doubtful to what extent the figures of Naegeli and Burkhardt are applicable to the population in general. In both these series the material was obtained in hospitals (Zürich and Dresden), and thus was selected. Further, as Flügge has correctly pointed out, the death-rate refers to all cases of death, children included, and does not refer to certain ages only. Calculating out Burkhardt's figures as percentage of healed and progressive tuberculosis to total deaths, Flügge makes out that the number of those "disposed" between the ages of 18—60 must approximate to 60—70 per cent., the number of non-disposed sinking to 30—40 per cent., a very different figure from that obtained by a superficial examination of Burkhardt's figures. Flügge points out further that, far from the tubercle bacillus being ubiquitous, it is in reality local, and thus the opportunity for infection must vary within wide limits. That a considerable number of human beings are tuberculous, however, is also shown by the inoculation of tuberculin carried out by Franz on the soldiers of two Austrian regiments. Over 60 per cent. gave a positive reaction.

The study of pathological anatomy in the last twenty years has gone to show more and more that the doctrine of a phthisical constitution is on a sound basis. It is impossible to analyse the publications dealing with this subject. Indeed, this is all the more unnecessary as it has been very completely done by Hart and Schlüter. This view is also supported by the work of Beneke, Birch-Hirschfeld, Schmorl, Bartel, and many others. The main problems studied have been in connexion with attempts to determine why the lungs, and especially the apices of these organs, are much more frequently affected than other parts of the body. Thus some have tried to uphold the doctrine of a general pulmonary disposition, others that there is an individual susceptibility of the apices on the basis of a general constitution. There certainly seems good ground for assuming that anomalies, congenital or acquired, of the upper thoracic aperture are responsible for frequent infections in this site.

Coming to work dealing more precisely with epidemiological and statistical inquiry, a large number of publications have made and are making their appearance. To notice these in detail would require more space than remains at our disposal; we shall, therefore, confine ourselves to a brief general review indicating what, in our judgment, are the salient features of different types of work. We can at once put on one side the very numerous lectures, addresses, and pamphlets which merely repeat evidence already discussed in earlier sections of this paper, or affirm conclusions (of most diverse natures) on the strength of general medical opinion, "universal experience," or the writer's (unpublished) observations. Such a process considerably reduces the amount of material to be examined. The residuum can be divided roughly into two classes: (1) Papers adducing evidence to prove that the environmental factor is the all-important one; (2) papers leading to a precisely contrary conclusion.

Work falling in Class 1 can again be divided in accordance with the method adopted into direct and indirect investigations. By direct investigations we understand papers in which facts are collected which are thought to demonstrate at once the all-sufficiency of the environmental factor. Most of these papers contain details of more or less extensive series of cases in which, according to the author, the hereditary factor seemed to be of little moment, or the existence of the disease in several members of a family could be referred to some common source of infection. A relatively favourable instance of this class of paper is afforded by a contribution published by v. Sokolowski in 1901. This author collected data respecting no less than 32,694 patients seen by him in private practice, including 8,074 tuberculous subjects. While recognizing the existence of an hereditary influence in certain cases, he was of opinion that its frequency was not so great as "die Aerzte es gewöhnlich annehmen." v. Sokolowski emphasized the magnitude of his series and called attention to the danger of basing conclusions upon but few instances.

In our opinion, however, v. Sokolowski's data are so presented that no conclusion whatever can be drawn from them with regard to the question of heredity. The sibships of the different individuals are not stated, nor the age distribution of the non-phthisical patients, so that it is impossible to say how many of them can reasonably be regarded as still "exposed to risk." The author's conclusion is, indeed, based upon a fallacy very common in medico-statistical writings. He seems to think that a knowledge of the frequency of tuberculosis among the ascendants of phthisical persons is a sufficient basis for deductions. It should be clear that to base a conclusion upon the single fact that in his series not more than 25 per cent. of the parents of tuberculous patients themselves suffered from the disease would be illogical, even if all the family histories were completed. The percentage of tuberculous ascendants in the stocks of non-phthisical patients was an essential preliminary, and this does not appear to have been ascertained; in fact, there is no evidence of a control experiment having been done. It is of interest to notice that the intermarital correlation, the only constant for the deduction of which v. Sokolowski provides sufficient data, is about 0·2 (Pearson and Pope, p. 8), a value indicating but little relationship.

We have dwelt upon v. Sokolowski's paper because it is a relatively favourable specimen of its class, the deductions not being of a very sweeping character. One cannot, however, fail to remark traces of a defective logical method which seems to us to vitiate so many similar results. In this connexion we may refer to the severe and, in our opinion, unanswerable strictures passed by Hamer upon the statistical evidence adduced by Cornet with regard to the Catholic Nursing Orders in Germany (Bulstrode).

It is not necessary to criticize a type of paper represented by the work of a German writer in which *three* cases of phthisis are described in two brothers and a sister. The author suggests that all may have been originally infected by the father, the disease being lighted up by sexual or other excesses, since the daughter had recently married, while of the two brothers, one, in particular, had exceeded "in Baccho et Venere."

Many writers have attempted to throw light upon the question by a study of the incidence of phthisis upon husbands and wives. As this subject was exhaustively discussed by Pearson and Pope in 1908, and was also the subject of a paper read by one of us at a meeting of this Section in 1909, we need not refer to the matter in detail. The conclusions expressed by the authors named, that interconjugal infection is a factor of but secondary importance, has not, we think, been seriously disputed; criticism having been mainly directed to the alleged imperfections of the data analysed.

On the whole, we venture to express the belief that no papers falling under the present category have materially added to the knowledge of thirty years ago, which has been detailed in earlier pages of this memoir. We accordingly turn to writers who have adopted a different and less direct process of inquiry.

During the last five-and-twenty years the tuberculosis-rate has markedly fallen in almost every, if not every, land. Within the same period a belief in the communicability of phthisis has been more and more disseminated. Much effort has been devoted to the diminution of possible sources of infection, attention has been directed to the necessity of disinfecting houses or rooms occupied by tuberculous patients, a war has been waged against the disgusting habit of indiscriminate expectoration, increasing numbers of phthisical patients have been segregrated in public institutions, and, generally, medical men and public health officials, both lay and professional, have laboured unceasingly to conquer the disease. It would naturally be expected that the fall in the phthisis-rate would be associated psychologically at least in many minds with the multifarious activities displayed. Such has been the case, and a considerable literature, both lay and professional, has sprung into existence. The fall in the tuberculosis-rate has, however, been differently interpreted by various authors. Some have laid stress upon the improved housing of the working classes, others have attached most importance to methods of disinfection, others to the influence of sanatoria and segregation. Newsholme, as the result of an elaborate inquiry, seemed to conclude that institutional segregation had been most powerful of the different factors, without, of course, neglecting other possible agents. Bulstrode and others have called attention to the difficulties of the problem, and among recent studies we would specially mention the dispassionate inquiry conducted by Professor Matthew Hay, of Aberdeen. Hay points out that the phthisis-rate was falling before any very serious measures had been collectively adopted to combat the disease. He remarks :—

> The decline began long before the germ origin of tuberculosis had been demonstrated by Koch, although the infectious character of the disease had never been without believers; but, what is more remarkable, the rate of decline was almost as great before any administrative attention was being paid to its infective character as it has been after such attention began. (Hay, p. 68.)

These facts give one pause and inevitably call to mind the fascinating subject of the rise, decline and fall of disease prevalence in instances not attributable to human agency. The epidemiological history of plague in England is a case in point.

We may further remark that the existence of a high correlation between the extent of, for example, institutional segregation and a fall in the phthisis-rate does not appear to us a fact of necessarily great significance. The difficulty that perpetually confronts the analyst of such data is caused by the existence of multiple correlation. The association between A and B may be indirect and dependent upon a common association with a third variable C. The theory of multiple correlation and its practical applicability have been so much extended by the labours of modern statisticians that at no distant time we may reasonably look for more light. It must, however, be remembered, that when more than a few variables are involved—and in the problem in question the number may not be small—arithmetical work becomes very laborious. In any case, whatever the future may have in store, no statistical proof of the all-sufficiency of any one of the possible factors or even of the predominating importance of any one, has yet, we think, been adduced. Some workers have realized the statistical difficulties of the problem. Speaking of infection in phthisis, Newsholme writes :—

> The evidence convincing one as to this does not lend itself to statistical statement, and when stated in print it may not be convincing; but the steady succession of cases in infected households at intervals of one or two years, or longer, the intercurrent destruction of children by tubercular meningitis or

"broncho-pneumonia" while their parents are suffering from chronic phthisis, and other similar evidence, can leave no doubt in the minds of those who come into continuous contact with the actual facts. (Newsholme, p. 70.)

With the addition of the words, "and are a priori convinced of the non-existence of a constitutional liability," this extract would not inaptly express the judgment we ourselves have been led to form. In sum, while the indirect method, the study of vital and sanatory statistics, has undoubtedly revealed the existence of phenomena well deserving further inquiry, it has not, we believe, substantially widened our knowledge of the particular problem which engages our attention.

Finally, we must examine recent contributions to the study of constitutional and hereditary factors. Here, again, a classification into direct and indirect methods can be made, but the number of papers falling within the latter sub-class is not great. Many, indeed most, of the writers who have emphasized the hereditary factor in phthisis seem to us to exhibit the same logical insufficiency which appeared to characterize writings of an opposed school, but a few papers are deserving of a fuller notice.

An attempt has been made, notably by Turban and Strandgaard, to obtain direct evidence of the hereditary transmission of a phthisical *habitus*. Turban published a series of cases in which members of the same family affected by phthisis exhibited a considerable amount of agreement in respect of the age at which the disease first appeared, the site of the lesion and the general course of the disease. From such data he argued that the members of a common stock resemble one another in the possession of a *locus minoris resistentiæ*, such peculiarity being the basis of an hereditary character in the disease. Since Turban did not publish, together with his family records, any data from which the relative frequency of primary disease in different lungs or regions of one lung could be deduced, it was difficult to assent to his conclusion. Quite recently Strandgaard has published another series of family observations, and at the same time provided somewhat extensive material from which the relative frequency of right pulmonary phthisis, left pulmonary disease, and their combinations in each stage (adopting Turban's classification into three stadia) could be calculated. Strandgaard himself arrived at substantially the same conclusion as Turban with regard to the main issue of an inherited *locus minoris resistentiæ*. If we classify pulmonary lesions into (1) mainly right lung affections, (2) mainly left lung affections, (3) cases in which both lungs are equally involved, then in a pair of phthisical patients taken at random six combinations are possible—viz., RR, LL, LR, LB, RB, BB, where R signifies class (1), L class (2) and B class (3). From a knowledge of the relative frequencies of the three classes in the general "population" of phthisical patients, it is evidently possible to calculate the probable frequency of each of the six combinations in a sample of pairs which might be expected to occur as a matter of chance. Evidently the same method can be extended to groups of three, four, &c. We found as a matter of fact, on applying this arithmetical test to pairs of brethren at the same stage of the disease, that the frequency of the combination RL was decidedly less than the calculated value, so far bearing out Strandgaard's interpretation of his material. The actual frequencies were, however, based on too small numbers for the conclusion to have much importance attached to it.

Time has not permitted of fully analysing the material, but our general impression is that a complete study is fraught with grave difficulties, and that this class of inquiry, although of interest, is not particularly hopeful. A judgment as to the exact localization and extent of the pulmonary lesion is based, in the large majority of instances, upon the results of physical examination. Not being clinical workers, we express an opinion on the value of the methods of diagnosis in current use with great diffidence; we think, however, that we are but echoing the opinions of highly skilled practitioners when we suggest that, however expert the observer, such results are subject to much error. That final court of appeal, the post-mortem table, has not always confirmed the decision of the clinical judge as to the extent or even the site of the pulmonary lesion. Apart from this, an examination of Strandgaard's paper will show that it is, in a fair percentage of the cases, difficult to decide which lung is chiefly involved, so that two independent classifications of the same material might lead to appreciably different results.

The English Biometric school have devoted much attention to the hereditary factor in phthisis. In 1908, Pearson analysed material from the Crossley Sanatorium; he concluded that the intensity of the relationship between the present or past existence of tuberculosis in the parent, and the same disease in the offspring, was of such an order that hereditary transmission of a tendency to develop the complaint must be assumed to exist. The values of the coefficients of correlation led Pearson to conclude that the inheritance of a phthisical predisposition, or, as he termed it, diathesis, was of the same order of intensity as had been found to characterize the transmission of normal physical characters in man. Pearson further expressed the opinion that this factor was of more importance in the genesis of the disease than ordinarily infective conditions (Pearson, p. 25). Similar conclusions were reached by Goring, who analysed statistics relating to 1,500 criminals.

These results have been subjected to considerable criticism, it having been urged in the first place that the correlation between parent and child might be a measure rather of infection than of true inheritance; in the second place, it was pointed out that data of this kind cannot conform to a high standard of accuracy. In a subsequent paper, Pearson argued that, were infection the chief factor, the correlation between husband and wife should be of the same order as between parent and child, but that it was in fact much smaller.

It appears to us that the second objection, viz., the alleged untrustworthiness of the statistics, has been to some extent met by the uniformly higher values of the ancestral correlations as compared with the inter-marital constants obtained from material of essentially the same class. It is difficult to understand how errors of record, apart from widespread and intelligent falsification, could lead to such a result. This impression is confirmed by the concordance of Goring's conclusions with those of Pearson, since the former's material was not subject to precisely the same kind of error as that analysed by Pearson, Pope, and Greenwood.

We should not, however, be prepared to regard a study of marital tuberculosis as fulfilling all the conditions of a true control, from the point of view of infective possibilities, since resistance to infection *may* increase in adult life,[1] although we are not acquainted with any convincing evidence in man that this is so. It will perhaps be thought that, while biometric studies have somewhat strengthened the evidence in favour of a belief in the importance of an hereditary factor, they have not materially altered the position of thirty-five years ago. These remarks must not be interpreted as implying any disparagement of the care and precision which distinguish the papers under notice.

Lastly, a word may be said as to indirect methods of approaching the subject. If it could be shown from a statistically adequate sample of persons suffering from tuberculosis that they differ significantly in such anthropological characters as skin and eye pigmentation, craniological constants, &c., from the population out of which they come, it would be fair to argue that the factor of inherited predisposition is of importance, since few will now deny the heritability of many such characters. Of work dealing with this aspect, the papers of Shrubsall may be mentioned. This author has found some association between brunette traits and pulmonary tuberculosis. It can, of course, be urged that work of this kind is difficult to interpret, since even were some such association definitely established, it might be indirect. It might be, for instance, that a certain type of physique is ill fitted to survive in the struggle for life, and that its possessor tends to be driven to those conditions of environment under which the chances of infection are maximal. Obviously, however, such a criticism is double edged. These anthropometric inquiries undoubtedly deserve further extension.

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[1] *See* Greenwood, pp. 7-10.

The reader will have gathered from what has gone before that, in our opinion, the many workers who have dealt with the problem of inheritance and infection during the last few years have failed to achieve any really solid advance from the classical position. Such a conclusion will, of course, have been anticipated by anyone who has contemplated the development of a science, noting the rapid progress up to a certain point, followed by advances each so small as to be inappreciable. Nevertheless, an attentive consideration of the facts relative to our knowledge of tuberculous disease, some of which we have endeavoured to review, would be of much benefit to those persons who say or imply that what has been done in our own time far exceeds in importance the combined efforts of all previous generations. Any such assertion is repugnant to sound scholarship, calculated gravely to mislead the general public, and an injury to the cause of medical science.

More than twenty-eight years ago Leichtenstern made the following remark in the first blush of enthusiasm following the isolation of the tubercle bacillus, and it remains substantially true to-day: "For all Koch's brilliant discovery there are still many lacunæ and open questions facing us in the ætiology of consumption, and these offer to the practitioner, to the statistician, to the pathologist who studies the history and geography, to the pathologist who experiments, and to the bacteriologist, a wide field wherein to co-operate."

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THE PROCESS OF DISINFECTION BY CHEMICAL AGENCIES AND HOT WATER.

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(With 21 Text Figures.)

CONTENTS.

PAGE

I. Introduction 238

II. Disinfection with phenol 240

1. Further experiments on disinfection with phenol of:
   - (*a*) *B. typhosus* 241
   - (*b*) *B. coli commune* 243
   - (*c*) *Staphylococcus pyogenes aureus* 244
2. General conclusions upon disinfection with phenol . . 249
3. Summary of Section II 251

III. Disinfection by hot water 252

1. General remarks upon disinfection by heat 252
2. Description of the method employed and of experiments made with:
   - (*a*) *B. typhosus* 254
   - (*b*) *B. coli commune* 257
   - (*c*) *B. paratyphosus* 260
   - (*d*) *Staphylococcus pyogenes albus* and *Staphylococcus pyogenes aureus* 261
   - (*e*) *B. pestis* 268
3. Temperature coefficient 268
4. Influence of acids and alkalis 272
5. Analogy between the disinfection of bacteria by hot water and the "heat coagulation" of proteins . . . 277
6. Summary of Section III 278

IV. Disinfection by sunlight and drying 280

V. Summary and general conclusions 281

I. INTRODUCTION.

Krönig and Paul (1897) were the first to study the process of disinfection by making quantitative observations at intervals during its progress. Their figures showed that disinfection proceeded in an orderly manner and that the rate diminished as the number of survivors became less. Ikéda (1897) attempted to express their results by an empirical formula.

In 1907—1908, Madsen and Nyman, and H. Chick working simultaneously but independently at this subject, found that during the process of the disinfection of anthrax spores by chemical agents (mercuric chloride and phenol respectively) the concentration of spores remaining alive varied logarithmically with the time, and that therefore the number of anthrax spores destroyed per unit time was proportional to the number present in a unit volume of the medium at that moment. An analysis of the figures published by Krönig and Paul showed the same relationship. Tables I*a* (H. C. 1908, p. 99) and I*b* (K. and P. 1897, p. 26) are examples of the progress of disinfection of anthrax spores with phenol and mercuric chloride respectively; the corresponding curves in Figs. 1*a* and 1 *b*, where logarithms of concentration of survivors are plotted against time, show the logarithmic relation graphically.

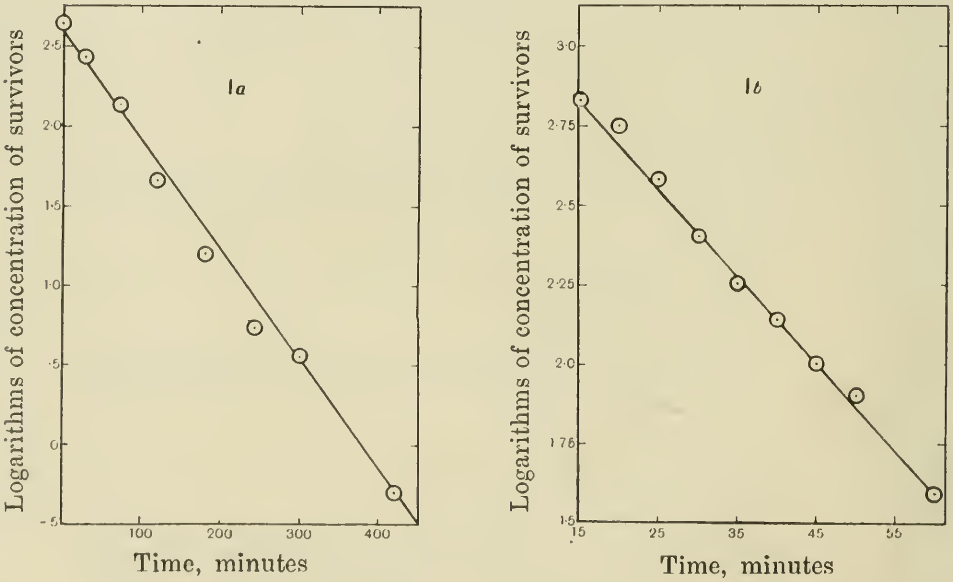

Fig. 1. (*a*) Disinfection of anthrax spores with 5 % phenol at 33·3° C. (H. C. 1908, Table II, p. 99).

(*b*) Disinfection of anthrax spores with 0·11 % mercuric chloride at 18° C. (Krönig and Paul, 1897, Table IX, p. 26).

TABLE I *a*.

Disinfection of Anthrax Spores with 5 % *phenol at* 33·3° C.

(See H. C. (1908), Table II, p. 99.)

Time, minutes $=t$	Mean number of bacteria surviving in one drop of disinfection mixture, $=N$	$\text{Log}_{10} N$	$^*K=\frac{1}{t_n-t_0}\frac{\log N_0}{\log N_n}$
$0=t_0$	$439=N_0$	2·642	—
30	275·5	2·439	·0068
75	137·5	2·138	·0067
120	46	1·663	·0082
180	15·8	1·199	·0080
246	5·45	·736	·0077
300	3·6	·556	·0069
420	0·5	– ·301	·0070

* In this and all subsequent tables, values of K from the expression $\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$ are calculated with Briggs' logarithms in place of natural logarithms.

TABLE I *b*.

Disinfection of Anthrax Spores with 0·11 % *mercuric chloride at* 18° C.

(See Krönig and Paul (1897), Table IX, p. 26.)

Time, minutes $=t$	Mean number of bacteria surviving $=N$	$\text{Log}_{10} N$	$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$
$10=t_0$	$2027=N_0$	3·307	—
15	672	2·827	·096
20	564	2·751	·056
25	382	2·582	·048
30	251	2·400	·045
35	179	2·253	·042
40	138	2·140	·039
45	101	2·004	·037
50	80	1·903	·035
60	39	1·591	·034
70	6	·778	·042
80	3	·477	·040

The conclusion arrived at by Madsen and Nyman and also by myself was that if the disinfectant be present in large excess relative to the bacteria, disinfection proceeds in the same manner as a reaction of the first order, that is to say, as a unimolecular reaction, or, what amounts to the same thing, as a bimolecular reaction with one reagent in excess.

The reason for it so doing must, apparently, be sought for in the same direction as in the case of the hydrolysis of sugar by water or the decomposition of hydrogen arsenide by heat, which are both reactions of the first order. The explanation offered by the physical chemist is that there is no permanent dissimilarity between the molecules, but that at any one time only a proportion of them is in the condition to undergo the dissociation or chemical union. This temporary increased susceptibility is believed to be occasioned by differences in the internal energy of the molecules. This interpretation has the advantage of affording a possible explanation of the influence of temperature upon the rate of reaction.

It would appear that with anthrax spores we are dealing with a population possessing but slight permanent differences in regard to their behaviour towards disinfectants, and that the fact that the whole number is not killed off in the same time is not, as has been supposed, due to permanent variations in resistance of individuals but, as in the chemical analogies given above, occasioned by temporary energy changes in the molecules of which the bacterial proteins are composed.

In the case of *B. paratyphosus*, the vegetative form worked with (H. C. *loc. cit.*), broth cultures, obtained by successive sub-culturing at frequent intervals, behaved as anthrax spores on disinfection with phenol and the nature of the disinfection process was similarly explained. The observations made upon 24 hours' cultures of *B. paratyphosus,* however, indicated the existence in this case of some disturbing factor which caused the process of disinfection to depart from the simple law found to apply in the case of spores and "young" cultures.

These interpretations, put forward in the previous paper already referred to, have been criticised by Eijkman (1909), and by Hewlett (1909) in his Milroy Lectures. Although these criticisms can, I think, be met by appeal to the facts already published, they have stimulated me to perform some further experiments. These experiments will be presented first and the points raised by my critics will be dealt with subsequently.

II. DISINFECTION WITH PHENOL.

(1) *Method employed.*

The method employed for studying the reaction velocity of disinfection in this instance was the same as that used in the previous paper (H. C. 1908). A measured quantity (five drops from a standard capillary pipette = ·08 c.c.) from a 24 hours' broth culture of the organism selected was added to a tube containing 5 c.c. phenol solution immersed in a water bath maintained at 20° C. This disinfection tube was fitted with a capillary pipette by means of which samples could be withdrawn and a definite number of drops taken for agar or gelatine plate cultures at successive intervals of time. Three plates were poured at each time of sampling, necessitating the help of two assistants. The sample drops were dropped into 0·5 c.c. of sterile distilled water, previously placed upon the plates; this lessened the error arising from the impossibility of pouring all three plates simultaneously. The plates were counted after 48 hours' incubation at 37° C. in the case of agar, or after four or five days' at 20° C. in the case of gelatine plates, and the progress of the disinfection reaction was studied by thus enumerating the surviving bacteria at successive periods of the process.

(*a*) *Experiments with B. typhosus.*

Tables II and III give the results of two experiments with *B. typhosus* and 0·6 % phenol at 20° C. Gelatine[1] plates were used in

TABLE II.

Disinfection of B. typhosus with 0·6 % *phenol at* 20° C.

Exp. 8. 1. '10.

Time, minutes $=t$	Amount of sample taken, drops	Numbers on plates (gelatine)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\mathrm{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0·5 = t_0	1	236 218 240	231·3	231·3 = N_0	2·364	—
1·5	1	188 215 167	190	190	2·279	·085
2·6	1	160 157 184	167	167	2·223	·067
4·6	2	215 242	228·5	114·2	2·058	·075
6·6	2	139 172 156	155·7	77·8	1·891	·077
10·5	3	100 93 91	94·7	31·6	1·500	·086
15	3	33 26 43	34	11·3	1·053	·090
20	5	14 14 8	12	2·4	·380	·102
30	10	4 3	3·5	·35	− ·456	·096
					Mean	·086

---

[1] Gelatine plates were substituted for agar in all the later experiments in spite of the obvious disadvantages. There is some risk of error in the use of agar owing to the necessarily high temperature at which the plates are poured, and comparative experiments showed that higher numbers were invariably obtained by using gelatine, although the proportion remained fairly constant.

TABLE III.

Disinfection of B. typhosus with 0·6 °/₀ *phenol at* 20° C.

Exp. 14. 1. '10.

Time, minutes $=t$	Amount of sample taken, drops	Numbers on plates (gelatine)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$
$0{\cdot}5=t_0$	1	779 717 645	714	$714=N_0$	2·854	—
1·5	1	584 571	577·5	577·5	2·762	·092
2·5	1	406 526 451	461	461	2·664	·095
4·5	1	313 215 288	272	272	2·435	·105
6·5	1	136 149 145	143·3	143·3	2·156	·116
10	2	47 162 155	121·3	60·6	1·782	·113
20	5	35	—	4·48	·651	·113
	10	23 54	—			
					Mean	·106

both experiments. Disinfection, as in the case of anthrax spores, proceeded so that the number of survivors in unit volume varied logarithmically with the time; the logarithms of these numbers, when plotted against time, also gave straight lines (Fig. 2). The velocity constant, $K\left(=\frac{I}{t_n-t_0}\log\frac{N_0}{N_n}\right)$, calculated from the experimental numbers, is given in the last column of either table, and shows fair constancy in value.

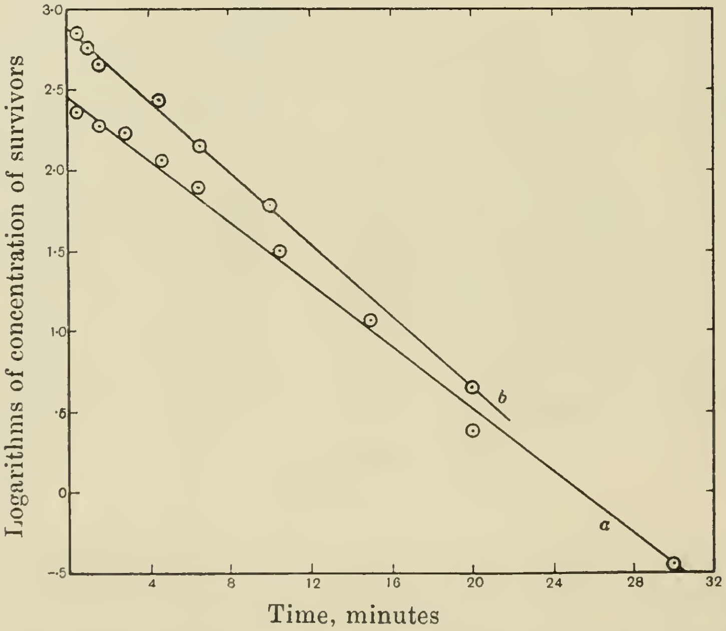

Fig. 2. Disinfection of *B. typhosus* with 0·6 °/₀ phenol at 20° C.
(*a*) Exp. 8. 1. '10, Table II. (*b*) Exp. 14. 1. '10, Table III.

(*b*) *Experiments with B. coli commune.*

Tables IV and V give the results of two experiments with *B. coli commune* and 0·5 % phenol at 20° C., in the one case agar and in the other gelatine plates being used. 24 hours' cultures in broth at 37° C.,

TABLE IV.

Disinfection of B. coli commune with 0·5 % phenol at 20° C.

Exp. 15. 2. '10.

Time, minutes $=t$	Amount of sample taken, drops	Numbers on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$
(0·8	1	593 623	608	608	2·784)	—
$1·9=t_0$	1	286 354 256	299	$299=N_0$	2·476	—
2·9	1	229 219 301	249·7	249·7	2·397	·079
4·25	1	193 209 217	206·3	206·3	2·314	·069
5·75	1	192 172 158	174	174	2·240	·061
7·5	1 2	127 261	— —	129·3	2·112	·065
10	2	144 207	175·5	87·75	1·943	·066
15	3 5	121 261	— —	47·7	1·678	·061
20	5 10	116 195	— —	20·73	1·317	·064
30	10	51 20	35·5	3·55	·550	·068
40	20	7 13	10	·5	−·301	·073
					Mean	·067

TABLE V.

Disinfection of B. coli commune with 0·5 % phenol at 20° C.

Exp. 21. 3. '10.

Time, minutes $=t$	Amount of sample taken, drops	Numbers on plates (gelatine)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$
(0·6	1	645 623 648	639	639	2·805	—
1·5	1	565 564 639	589	589	2·770)	
$2·5=t_0$	1	436 470 475	460	$460=N_0$	2·663	—
3·75	1	425 395 364	395	395	2·597	·053
5	1	340 329 351	340	340	2·531	·053
7	2	493 513 498	501	250	2·398	·059
10	2 10	387 416 2080 1830	— —	196	2·292	·049
15	2	204 273	238	119	2·075	·047
20	20	1670 1780	1725	86·2	1·935	·042
					Mean	·051

made from a stock agar culture grown at room temperature, again served as the material to be disinfected and the method was exactly as above described.

The results are similar to those obtained in the case of *B. typhosus*; as disinfection proceeds a logarithmic relation is approximately maintained between the time of disinfection and the concentration of surviving bacteria.

In some cases for a very short period (usually less than two minutes) at the beginning of the experiment there was some irregularity. Numbers counted during this period are not plotted in Fig. 3 or used for calculating the velocity constants in Tables IV and V. I am inclined to attribute this to a difficulty with this species in obtaining perfect mixing and accurate sampling during the first moments of an experiment. The variation was not consistent in one sense, in some cases the number of bacteria counted was larger, in others smaller than was to be expected. Eijkman's (1909) results which differ from the above will be referred to later (see p. 249).

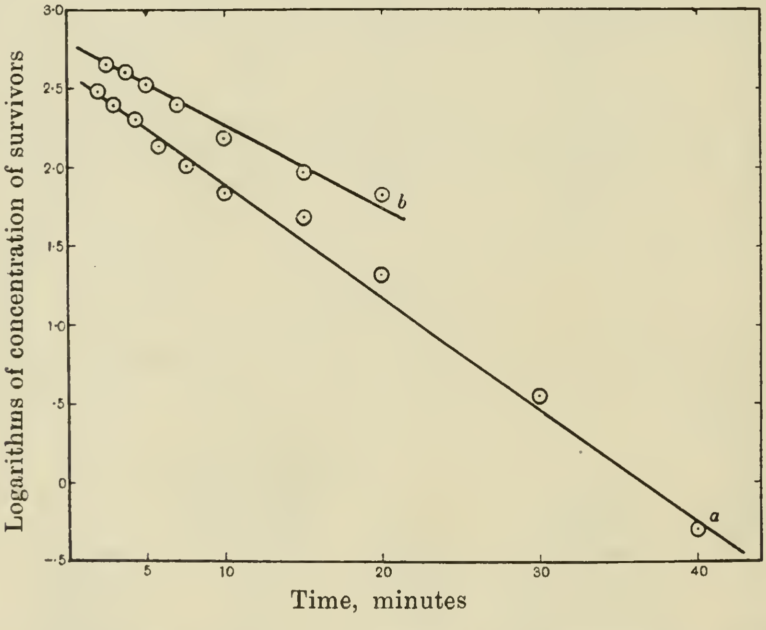

Fig. 3. Disinfection of *B. coli commune* with 0·5 $^0/_0$ phenol at 20° C.
(*a*) Exp. 15. 2. '10, Table IV. (*b*) Exp. 21. 3. '10, Table V.

(*c*) *Staphylococcus pyogenes aureus.*

Disinfection proceeds logarithmically except for a period at the beginning, during which disinfection is extremely slow. This period of lag lasted in all cases for four minutes, see Figs. 4 and 5, and was consistently present in contradistinction to the irregularity frequently noticed in the case of *B. coli commune*. At the end of this period disinfection proceeded in approximate accordance with the logarithmic law.

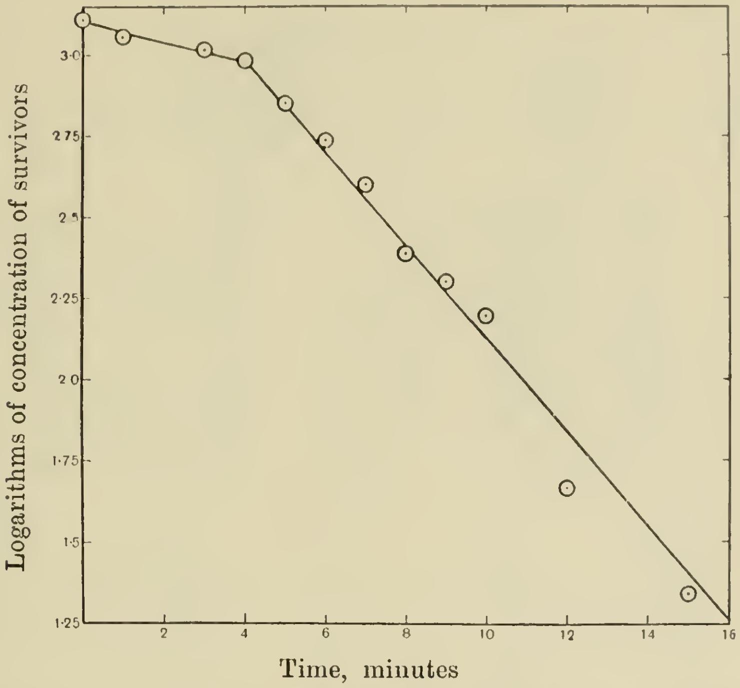

Fig. 4. Disinfection of *Staphylococcus pyogenes aureus* with 0·6 % phenol at 20° C. (Exp. 6. 4. '09, Table VI.)

TABLE VI.

Disinfection of Staphylococcus pyogenes aureus with 0·6 % *phenol at* 20° C.

EXP. 6. 4. '09.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
Control	1	1369 1218	1293	1293	3·112	—
1	1	1160 1120 1143	1141	1141	3·057	—
3	1	974 1064 1093	1044	1044	3·019	—
$4 = t_0$	1	986 929 940	952	$952 = N_0$	2·979	—
5	1	693 669 762	708	708	2·850	·129
6	2	1062 1048 1148	1086	543	2·735	·122
7	3	1253 1167 1187	1202	401	2·603	·125
8	3	728 731	729·5	243·2	2·386	·148
9	5	1113 912	1012·5	202·5	2·306	·135
10	5	761 691 890	780·7	156·1	2·193	·131
12	10	626 326 410	454	45·4	1·657	·165
15	10	220 216	218	21·8	1·338	·149
					Mean	·136

TABLE VII.

Disinfection of Staphylococcus pyogenes aureus with 0·6 $^0/_0$ *phenol at* 20° C.

Exp. 16. 3. '09.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K=\dfrac{1}{t_n - t_0}\log\dfrac{N_0}{N_n}$
Control	1	1222 1140	1180	1180	3·072	—
1	1	1005 978 1011	998	998	2·999	—
2	1	958 995 944	966	966	2·985	—
3	1	794 878 889	854	854	2·931	—
$4=t_0$	2	1498 1400 1570	1489	$744=N_0$	2·872	—
5·1	3	2039 1882 1649	1857	619	2·792	·073
7	8	2723 2586 2100	2469	309	2·490	·127
10	10	961 1172 1550	1228	122·8	2·089	·130
15	10 6	315 247 187	— —	29·1	1·464	·128
					Mean	·114

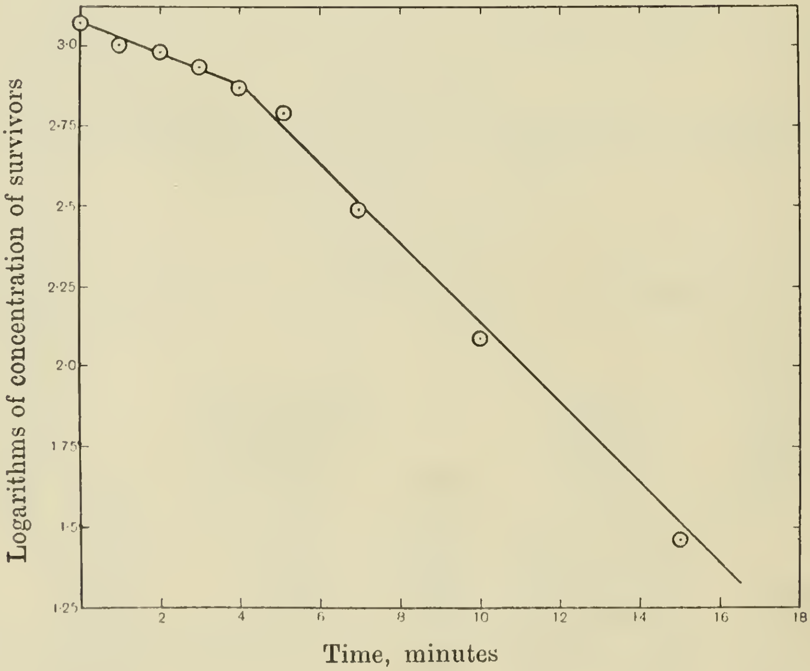

Fig. 5. Disinfection of *Staphylococcus pyogenes aureus* with 0·6 $^0/_0$ phenol at 20° C. (Exp. 16. 3. '09, Table VII.)

The results of two experiments with *Staphylococcus py. aur.* and 0·6 $^0/_0$ phenol at 20° C. are given in Tables VI and VII. For reckoning the value of the velocity constant in these tables the initial concentration of bacteria is taken as that obtaining at the end of the "period of lag," as determined from the curves in Figs. 4 and 5 respectively, where logarithms of concentration of survivors are plotted against time. The results of two additional experiments are given in Table VIII.

TABLE VIII.

Disinfection of Staphylococcus pyogenes aureus with 0·6 % *phenol at* 20° C.

Exp. I, 30. 3. '09.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
3	1	310	310	310	2·491	—
$4 = t_0$	1	284 224 236	248	$248 = N_0$	2·394	—
5	1	120 128 127	125	125	2·097	·30
6	1	107 109	108	108	2·033	·18
7	2	95 88	91·5	45·7	1·660	·24
8	2	63 47	55	27·5	1·439	·24
9	2	36 36	36	18	1·255	·23
10	3	13 14 33	20	6·66	0·823	·26
12	5	26 25	25·5	5·1	·708	·21
15	5	17 19 18	18	3·6	·556	·17
					Mean	·23
Exp. II, 13. 3. '09.						
1	1	663 641 633	646	646	2·810	—
$3 = t_0$	1	246 260 254	253	$253 = N_0$	2·403	—
5	1	42 49 41	44	44	1·643	·38
7	2	40 24 20	28	14	1·146	·31
10	2	4 6 7	5·66	2·83	·452	·28
					Mean	·32

In Table IX are given the results of two experiments with *Staphylococcus py. aur.* and 0·6 % phenol, in which comparison is made between the disinfection of bacteria grown at 37° C. (Exp. *a*) and bacteria grown at 42° C. (Exp. *b*), the conditions of experiment being otherwise identical. Disinfection in the first case was consistent with the experiments described above, but the bacteria grown at the higher temperature displayed as a whole a greatly increased resistance, and disinfection was so slow that it was impossible to study it within the time of the experiment. A similar phenomenon was noticed in the case of disinfection of *B. typhosus* with phenol by Rideal and Walker (1903, p. 431), and by Martin and the author (1908, p. 661).

TABLE IX.

Disinfection of Staphylococcus pyogenes aureus with 0·6 °/$_0$ *phenol at* 20° C.

Experiments to show difference in resistance of a culture grown at 37° C. and one grown at 42° C.

Exp. 23. 3. '09. (*a*) Culture grown at 37° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
Control	1	656 679	667	667	2·824	—
1	1	600 709 554	621	621	2·793	—
2	1	659 616 516	597	597	2·776	—
3	1	587 616 613	605	605	2·782	—
$4=t_0$	1	510 544 460	505	$505=N_0$	2·703	—
5	2	765 932	848	424	2·627	·076
7	3	1115 847 1017	993	331	2·520	·091
10	10 8	1488 1410 1317	— —	150·5	2·177	·088
					Mean	·085

(*b*) Culture grown at 42° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	K
Control	1	116	116	116	2·064	—
2	1	80 102 101	94·3	94·3	1·975	—
3	1	117 91	104	104	2·017	—
5	2	171 190 156	172·3	86·1	1·935	—
7	3	231 255 265	250	83·4	1·921	—
10	10	825 768 843	812	81·2	1·910	—

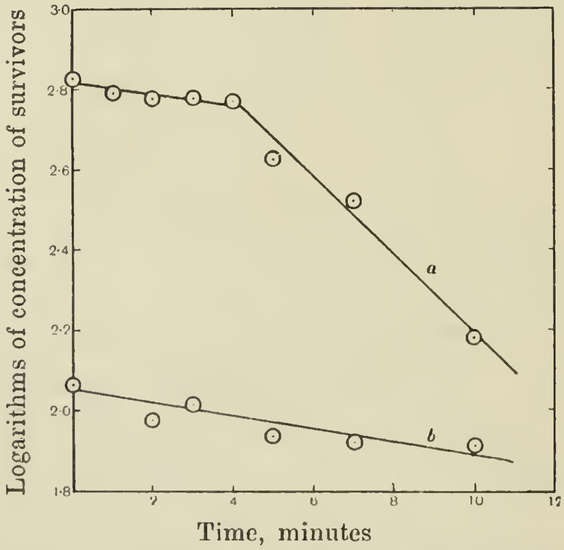

Fig. 6. Disinfection of *Staphylococcus pyogenes aureus* with 0·6 °/$_0$ phenol at 20° C. (Table IX.)

(*a*) 24 hours' culture at 37° C. (*b*) 24 hours' culture at 42° C.

(2) *General conclusions upon disinfection with phenol.*

Disinfection with phenol may in general be considered to proceed in accordance with a logarithmic law so that the concentration of survivors varies logarithmically with time, or, in other words, the rate of disinfection at any moment is proportional to the concentration of surviving bacteria $\left(-\frac{dn}{dt}=Kn\right)$.

Of the species worked with, anthrax spores, *B. typhosus*, and cultures of *B. paratyphosus* obtained by successive sub-culturing after short periods of growth, may be cited as proof of this assertion, without further remark. In my opinion, *B. coli commune* and *Staphylococcus pyogenes aureus* (if we allow for a short "period of lag" at the beginning of disinfection) may be added to this list.

Eijkman, however, came to the conclusion that the rate of disinfection of *B. coli commune* by phenol (1909) or hot water (1908) was not proportional to the concentration of survivors, except possibly for a short period in the middle of the disinfection, and that a much slower rate obtained not only at the beginning but also at the end. The "period of lag" constantly present at the start was called by him a "period of incubation." This is contrary to my own experience of *B. coli*, which, whether disinfected by phenol or by heat (see below), I have found to die at a rate approximately proportional to the concentration of survivors. Slight discrepancies occur at the beginning of disinfection, but these, I think, are attributed to error in sampling. My experience confirms that of Eijkmann (1908, p. 17, and 1909, p. 6), who had great difficulty in obtaining uniformity in cultures of *B. coli*.

In the case of *Staphylococcus* and 24 hours' cultures of *B. paratyphosus* the departure from the logarithmic law at the beginning of disinfection is of a different character. The *invariable* "incubation period" in the first case, and the *invariable* preliminary rush in the second, are evidently realities and must be attributed to some idiosyncracy of the species in question. With *Staphylococcus*, after about 4 minutes, disinfection proceeds in an orderly manner so that the delay is possibly due to a slow permeability of the bacterial envelope to phenol.

In the case of 24 hours' cultures of *B. paratyphosus* (see H. C. 1908, p. 109) the surviving bacteria decrease in number more rapidly at first than would be the case if the rate of disinfection were simply proportional to the concentration of bacteria at the moment, and a suggested explanation has already been put forward. This explanation has been criticised in the meantime by Hewlett (1909), so that a few further remarks may be permitted. The departure from the logarithmic law was found to be minimised by experimenting with material obtained by successively sub-culturing a small amount of a broth culture into fresh broth every 2—3 hours; in this case disinfection proceeded in a more regular manner and at the slower rate. This is shown in Fig. 7, which illustrates two experiments with *B. paratyphosus* and 0·6 % phenol (*loc. cit.* Tables VII and XII), one made with a 24 hours' culture and the other with material which had been successively sub-cultured three times at three hours' intervals. In the former case logarithms of concentration of living bacteria, plotted against time, give a curve; in the latter case, however, disinfection is more nearly logarithmic throughout, the experimental points lie on a straight line and the velocity constant has an approximately constant value.

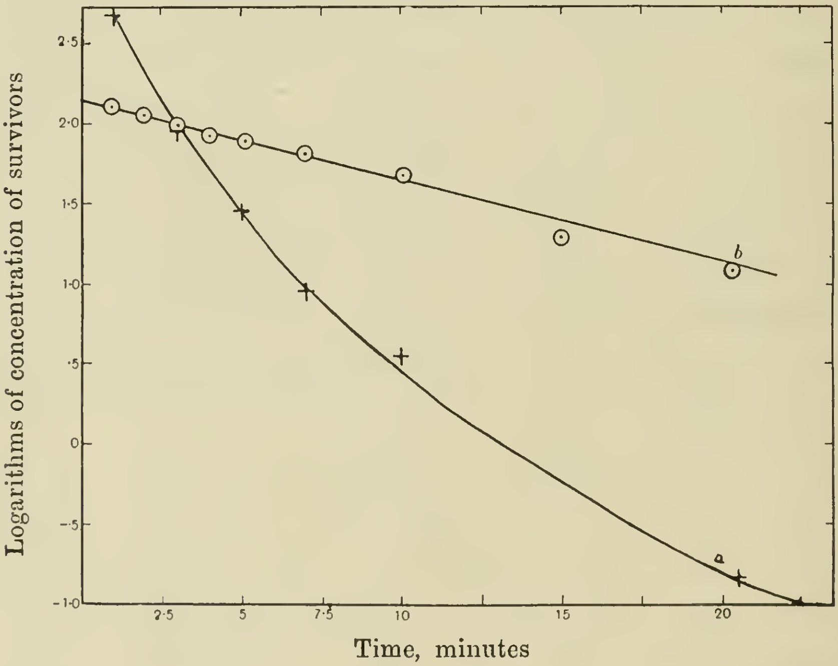

Fig. 7. (*a*) Disinfection of 24 hours' culture of *B. paratyphosus* with 0·6 % phenol at 21° C. (H. C. 1908, Table VII.)

(*b*) Disinfection of 3 hours' culture (3rd generation of 3 hours' cultures) of *B. paratyphosus* with 0·6 % phenol at 20° C. (H. C. 1908, Table XII.)

Hewlett (*loc. cit.* p. 50), after an analysis, among others, of the tables from which these curves are drawn, "can only see a slight balance in favour of the 'young' cultures giving a constant less variable than the constant obtained with 24 hours' cultures." Hewlett's treatment of these data is not quite legitimate. The tables he compares are not strictly comparable, the times at which samples were taken being different in the different cases. Comparison of curves, on the other hand, in which logarithms of concentration of surviving bacteria are plotted against time, is unexceptionable. From experiments with 24 hours' cultures of *B. paratyphosus* a series of curved lines are obtained, whereas from experiments with successively cultured material the lines are approximately straight, see Fig. 7.

The following interpretation was suggested to account for this apparent anomaly. Whereas in the case of anthrax spores and other species, where disinfection proceeds logarithmically, we may suppose temporary differences in resistance to disinfectants to exist among the individual bacteria (see also pp. 240 and 282), in 24 hours' cultures of *B. paratyphosus* we must suppose in addition the existence of permanent differences in resistance among the individuals, these differences to be in some way bound up with the "age" of the culture.

In my previous paper this was perhaps not very satisfactorily expressed, which may account for some misunderstanding. The age of individuals, as long as multiplication is regularly maintained during growth of a culture, would probably be the same in all cultures of whatever history, provided the temperature of incubation were the same. But a difference might exist between the population of a 3 hours' and a 24 hours' culture, owing to the fact that in the latter case the culture medium had altered in composition by the time the last multiplication had taken place, as a result of the large amount of growth it had already sustained[1]. In such a sense one may imagine differences to exist between "old" and "young" cultures. Whatever may be the correct manner of expression, it seems that the majority of the individuals in 24 hours' cultures of *B. paratyphosus* have a permanently weakened resistance to disinfectants, which must be distinguished from, and which they possess in addition to, those temporary differences in resistance by which I account for the logarithmic nature of disinfection in the ideal case.

(3) *Summary of Section II.*

1. Further experimental evidence is adduced to show that disinfection with phenol proceeds in accordance with a logarithmic law,

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[1] In my experiments 24 hours' cultures would seem to have attained the limit of growth, from the approximately constant number of bacteria they contained.

in a manner analogous to that of a reaction of the first order, so that the velocity of disinfection at any moment is proportional to the concentration of surviving bacteria $\left(-\frac{dn}{dt}=Kn\right)$.

Experiments have been made with

(*a*) *B. typhosus*,
(*b*) *B. coli commune*,
(*c*) *Staphylococcus pyogenes aureus*,

and compared with those made with anthrax spores and *B. paratyphosus*, which have been published previously.

2. In the case of *Staphylococcus py. aur.* there is invariably a short period (4 minutes with 0·6 % phenol) at the beginning of disinfection, when the disinfectant has little or no effect, after which disinfection proceeds in the normal manner. The suggestion is made that it is due to time taken for penetration of the bacterial envelope by the disinfectant.

3. In the case of 24 hours' cultures of *B. paratyphosus*, where disinfection proceeds at first at a rate which is in excess of what it would be if proportional only to the concentration of survivors, the majority of the individuals possess a permanently lessened resistance to the disinfectant. It is suggested, in amplification of a suggestion put forward in a previous paper, that this may be caused by exhaustion of the medium in which the bacteria have been grown. Cultures produced by successive sub-culture after short intervals of time show approximate conformity to the logarithmic law.

III. THE PROCESS OF DISINFECTION BY HOT WATER.

(1) *General remarks upon disinfection by heat.*

In disinfection by heat it is possible that three entirely different processes may be at work:

(1) the direct effect of heat upon the proteins of the bacteria,

(2) the effect, possibly hydrolytic, of water upon these proteins at high temperatures,

(3) desiccation of the organisms.

In sterilisation of bacteria by dry heat a combination of (1) with (2) or possibly with (2) and (3) may occur. The germicidal action of hot water at temperatures of 45° C. to 55° C., however, presents a very suitable subject for the study of the simplest disinfection process. For, from the analogous behaviour of proteins and enzymes, when treated with hot water, it would appear to present an uncomplicated instance of the second effect.

Madsen and Nyman (1907) investigated the rate at which anthrax spores dried in the air upon the surface of garnets (method of Krönig and Paul, 1897) were killed when placed in glass tubes and immersed in an oil bath at 100° C. and 110° C. respectively. At intervals one tube of garnets was removed, the garnets shaken in a measured quantity of water and plate cultures made. Their observations do not afford convincing evidence that, under these conditions, disinfection proceeds in accordance with a logarithmic law, but the authors, considering the roughness of the experimental method, seem satisfied with the agreement between calculated and observed values.

The following experiments refer to the effect of water at temperatures from 45° C. to 55° C., this method of experiment having been adopted in order to avoid some of the complications mentioned above.

(2) *Method employed.*

A wide mouthed test tube, capacity about 50 c.c., was fitted with a cork having two holes, into each of which a small piece of glass tubing had been fitted. The smaller glass tube *A* (see Fig. 8) acted as a bearing for the stirrer *C* which was attached to a bicycle hub by a

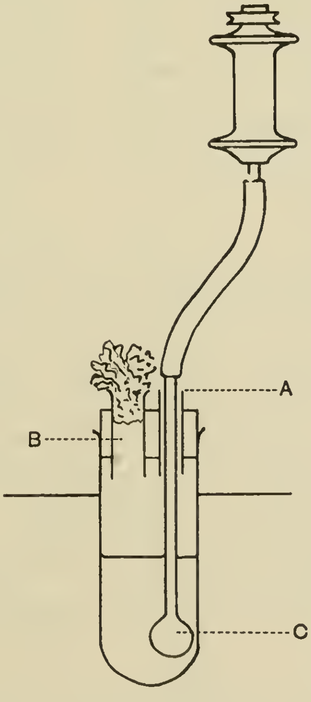

Fig. 8.

piece of india-rubber tubing. The bicycle hub had a small pulley at its upper end round which a cord passed to the shafting. The second tube, *B*, closed with a cotton wool plug, was used for adding the bacteria and for the withdrawal of the samples.

The whole apparatus being previously sterilised, 20 c.c. of sterile distilled water was placed in the tube and the whole allowed to remain in the bath sufficiently long to acquire the necessary temperature. A 24 hours' broth culture of the particular organism, grown at 37° C., was diluted about five to ten times, and at a given moment a small quantity of the diluted culture was introduced through the small tube *B*. At suitable times samples were withdrawn with sterilised pipettes, placed in separate small sterilised tubes and allowed to cool immediately.

At the conclusion of the experiment, plates were poured with measured quantities of the liquid in the series of tubes, smaller quantities (0·02 c.c.) from the earlier samples and larger quantities (0·2 to 0·4 c.c.) from the later samples.

The experiments were preferably so arranged that the disinfection tube contained about 500 to 2000 organisms per standard drop (25,000 to 100,000 per c.c.) at the start and samples were usually taken until there were less than 10 per drop.

The character of the disinfection process and its velocity at different temperatures was studied in the case of the following organisms:

(*a*) *B. typhosus*,
(*b*) *B. coli commune*,
(*c*) *B. paratyphosus*,
(*d*) *Staphylococcus pyogenes albus* and *Staphylococcus pyogenes aureus*,
(*e*) *B. pestis*.

(*a*) *Experiments with B. typhosus.*

Experiments with 24 hours' cultures of *B. typhosus* were made over a series of temperatures from 49° C. to 54° C. The results of two experiments at 54·15° C. and 49° C. respectively are given in Table X and graphically expressed in Fig. 9; the results of two further experiments at 52° C. and 52·1° C. are given in Table XI.

Disinfection proceeded according to the usual rule, approximately constant values were obtained for the velocity constant $K\left(=\frac{1}{t_n - t_0}\log\frac{N_0}{N_n}\right)$ and approximately straight lines were obtained on plotting time against logarithms of numbers of survivors present in unit volume. The first enumeration in the experiment at 49° C. (2008 bacteria per drop at 0·28 minutes) is out of line with the other determinations, and has been disregarded on the ground that, owing to imperfect mixing in so short a time, much stress should not be laid upon the result of the analysis of a sample taken during the first 20 seconds after addition of the bacteria. If this determination is included and used in the calculation of the values for the velocity constant, disinfection will apparently proceed at an excessive rate during the first minute ($K = {\cdot}308$). For the reason given above it is hard to say whether the phenomenon is a reality or not. In other experiments there is, however, also a fall, though slight, in the value of K as disinfection proceeds, see Exp. II, Table X, and Exps. I and II, Table XI.

TABLE X.

Disinfection of B. typhosus with hot water.

Exp. I, 9. 6. '09. Temperature 49° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0·28	1	1881 2122 2022	2008	2008	3·303	—
$1{\cdot}0 = t_0$	1	1298 1098	1198	$1198 = N_0$	3·078	—
2·05	1	914 769 1093	925	925	2·966	·107
3	3	1782 2246 2770	2266	755	2·878	·100
4	4	1947 2522 2038	2169	542	2·734	·114
5	1	449	—	488	2·688	·097
	10	5180 4640	—			
7	6	1788	—	289	2·461	·103
	10	2800 2940	—			
10	10	1113 1067 1204	1128	112·8	2·052	·114
15	10	211	—	24·2	1·384	·113
	20	514	—			
20	9	30	—	3	·477	·137
	10	28	—			
	20	51	—			
					Mean	·111

Exp. II, 9. 6. '09. Temperature 54·15° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0·25	1	509 444 472	475	$475 = N_0$	2·677	—
0·5	1	198 201	199·5	199·5	2·300	1·51
1·0	3	121 86 108	105	35	1·544	1·51
1·5	5	40 54 46	46·6	9·3	·968	1·37
2·0	10	16 18 13	15·66	1·566	·195	1·42
					Mean	1·45

It may therefore be said that with disinfection of *B. typhosus* by hot water an approximately logarithmic relation exists between time and concentration of surviving bacteria, and that any departure is in the direction of disinfection being quicker at the beginning and slower at the end of the process than would be the case if the agreement were complete.

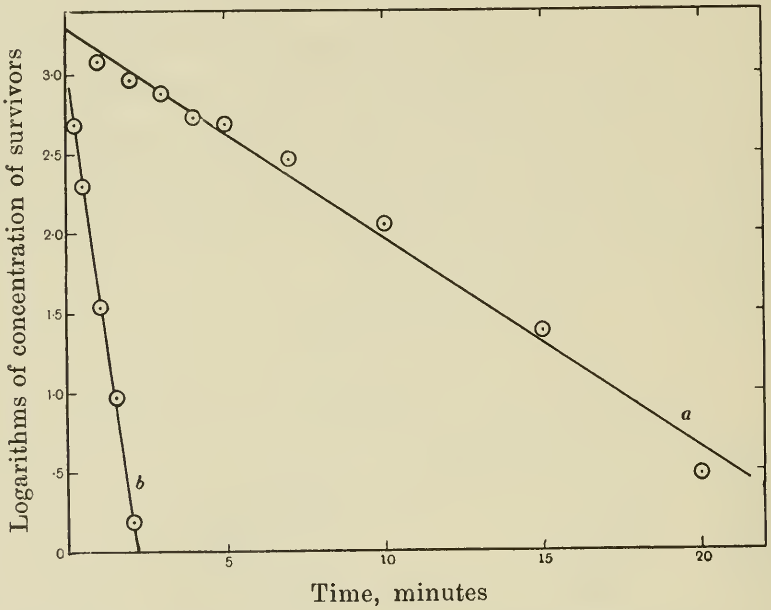

Fig. 9. Disinfection of *B. typhosus* with hot water (Table X).
(*a*) Exp. I at 49° C. (*b*) Exp. II at 54·15° C.

TABLE XI.

Disinfection of B. typhosus with hot water.

Exp. I, 2. 6. '09. Temperature 52° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$
0·25	1	204 180	192	$192=N_0$	2·28	—
1	1	6 29 20	18·3	18·3	1·26	1·36
2	2	3 0 4	2·33	1·16	·06	1·27
					Mean	1·31
Exp. II, 5. 6. '09. Temperature 52·1° C.						
0·29	1	576 470	523	$523=N_0$	2·718	—
1	2	189 251	220	110	2·041	·95
2	5	91 67	79	15·8	1·199	·89
4	10	18 15	16·5	1·65	·217	·67
					Mean	·84

(*b*) *B. coli commune.*

Experiments made with *B. coli commune* at temperatures from 49° C. to 53° C. gave results similar to those with *B. typhosus*.

Table XII and Fig. 10 express the results of two experiments using agar plates; Table XIII and Fig. 11 are from experiments made with gelatine plates.

The logarithmic relation existing between concentration of survivors and time of disinfection is subject to some slight irregularity. In Exp. I, Table XII, the value of K shows a general tendency to decrease,

TABLE XII.

Disinfection of B. coli commune with hot water.

Exp. I, 15. 2. '10. Temperature 48·9° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$0{\cdot}25 = t_0$	1	371 362 383	372	$372 = N_0$	2·570	—
1·0	1	266 253 304	274·3	274·3	2·438	·176
2	1	191 178 201	190	190	2·279	·166
4	1 2	68 76 129	— —	68·25	1·834	·196
6	1 2	25 44	— —	23	1·362	·210
10	2 5	16 29 67	— —	12·4	1·093	·152
15	5 10	10 15 18	— —	1·72	·235	·159
20	10 20	1 6 10	— —	·425	– ·372	·149
					Mean	·172
Exp. II, 15. 2. '10. Temperature 52·7° C.						
$0{\cdot}25 = t_0$	1	416 331 402	383	$383 = N_0$	2·583	—
1·0	1	207 235 183	208	208	2·318	·353
2	1	93 73 103	89·7	89·7	1·953	·360
4	2	21 47 19	29	14·5	1·161	·379
6	3 5	4 3 5	— —	1·1	·041	·442
8	10	6 5	5·5	·55	– ·260	·367
10	10	1 0 1	·66	·066	– 1·181	·386
					Mean	·381

TABLE XIII.

Disinfection of B. coli commune with hot water.

Exp. I, 26. 2. '10. Temperature 49° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (gelatine)	Mean.	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$0·75 = t_0$	1	341 375 276	331	$331 = N_0$	2·520	—
1·5	1	313 307 332	317	317	2·501	·025
2·5	1	249 282 295	275	275	2·439	·046
4	2	500 534 462	499	249·5	2·397	·038
6	5	922 868 834	875	175	2·243	·053
9	10	1740 1710 1490	1650	165	2·217	·037
14	20	1440 1540 1290	1420	71	1·851	·050
20	20	832 836 704	791	39·5	1·597	·048
					Mean	·042

Exp. II, 26. 2. '10. Temperature 52·5° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (gelatine)	Mean.	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$0·25 = t_0$	1	388 443 374	402	$402 = N_0$	2·604	—
0·75	1	360 411 388	386	386	2·587	·034
1·5	1	309 290 268	289	289	2·461	·114
2·5	2	478 469 493	480	240	2·380	·099
4	5	617 593 598	602·7	120·5	2·081	·139
6	13	812	—	63·8	1·805	·139
	20	1260 1310	—			
8	20	574 647 697	639	32	1·505	·142
					Mean	·111

in Exp. II and in both experiments in Table XIII to increase as disinfection proceeds. The latter type bears a resemblance to the experiments of Eijkman (see above, p. 249). All, however, conform more nearly than his to what I may call the ideal case, and, considering that the deviation is small, and not consistently in one direction, and that the species is moreover extremely variable, I conclude that here also disinfection proceeds so that the rate is approximately proportional to the concentration of survivors, and that straight lines most satisfactorily express the relation between the experimental points when logarithms of the latter are plotted against time.

The variability of this species is well shown by a comparison of Tables XII and XIII, where rate of disinfection of apparently similar material, at approximately the same temperature, is in the one case about four times as great as in the other.

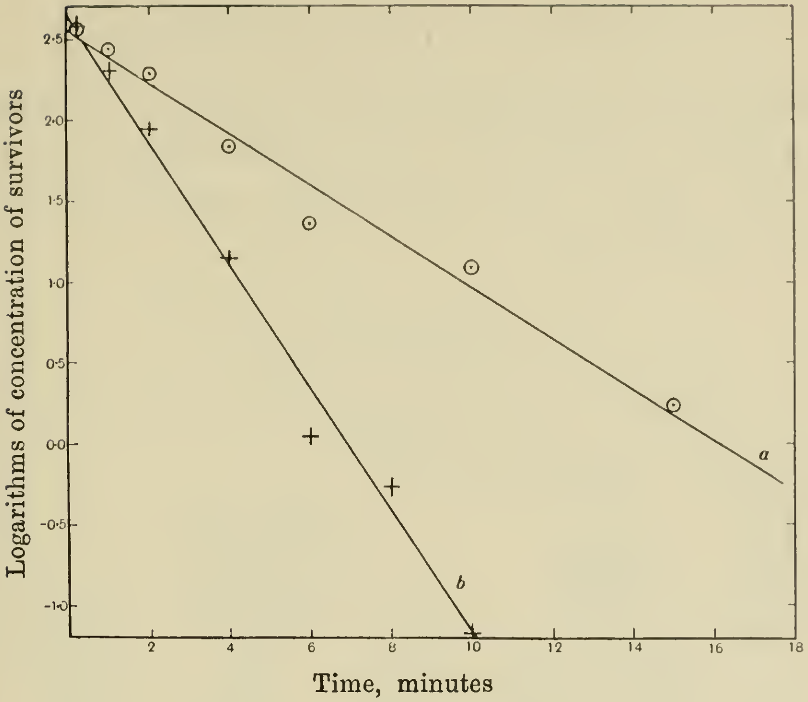

Fig. 10. Disinfection of *B. coli commune* with hot water (Table XII).
(*a*) Exp. I at 48·9° C. (*b*) Exp. II at 52·7° C.

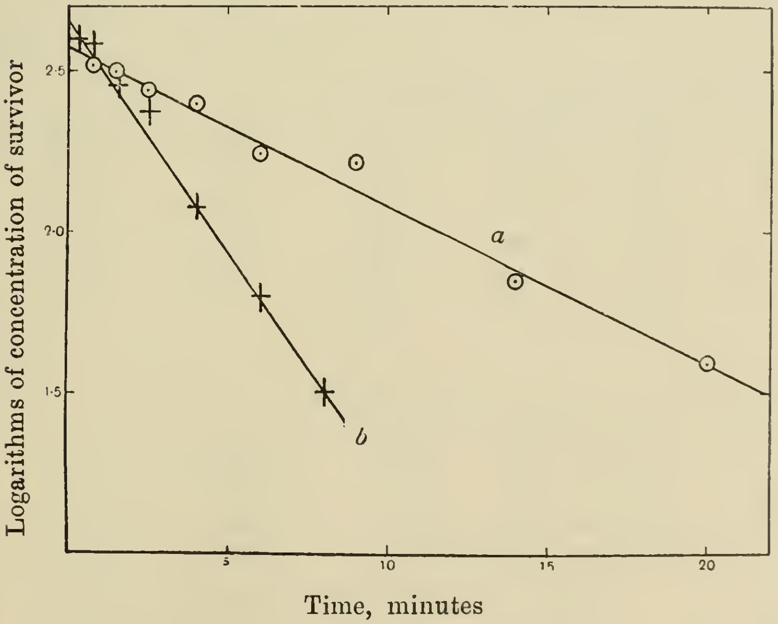

Fig. 11. Disinfection of *B. coli commune* with hot water (Table XIII).
(*a*) Exp. I at 49° C. (*b*) Exp. II at 52·5° C.

(*c*) *Experiments with B. paratyphosus.*

Experiments were made with 24 hours' cultures of *B. paratyphosus* at a temperature of 47·2—47·3° C., at which temperature the rate of death is fairly rapid. The results of three experiments are given in Table XIV and Fig. 12.

TABLE XIV.

Disinfection of B. paratyphosus with hot water.

Exp. I, 5. 5. '09. Temperature 47·2° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$
1·05	1	1117 1119 1143	1126	1126	3·051	—
2·2	1	774 824 836	811·3	811·3	2·909	—
$3=t_0$	1	672 639 694	668	$668=N_0$	2·825	—
5	1	441 487	464	464	2·666	·079
7	2	673 684 631	662·7	331·3	2·520	·076
10	3	618 580	599	199·7	2·300	·075
15	5	457 397 412	422	84·4	1·926	·075
20	5 10	198 408 502	— —	44·3	1·646	·069
					Mean	·075
Exp. II, 23. 4. '09. Temperature 47·2° C.						
$10=t_0$	1	307 242 248	266	$266=N_0$	2·425	—
15	1	102 133 83	106	106	2·025	·080
20	2	104 107 105	105·3	52·6	1·721	·070
25	3	94 85 104	94·3	31·4	1·497	·061
30	4	50 61 47	52·66	13·16	1·119	·065
41	5	16 12 19	15·66	3·13	·495	·062
					Mean	·068
Exp. III, 27. 4. '09. Temperature 47·35° C.						
$7=t_0$	1	1799 1825 1805	1810	$1810=N_0$	3·258	—
10	1	1220 1313 1241	1258	1258	3·100	·053
13	2	1296 1333 1343	1324	662	2·821	·073
16	3	1125 1267 1261	1218	406	2·608	·072
26	6 10	456 717 783	— —	75·2	1·876	·073
32	10	247 265 249	253·7	25·37	1·404	·074
					Mean	·069

After the first few minutes the accordance with the logarithmic law is very good, but, as was found to be the case with chemical disinfectants, the reaction proceeds more rapidly at first than would be the case were the rate dependent only upon the concentration of surviving bacteria.

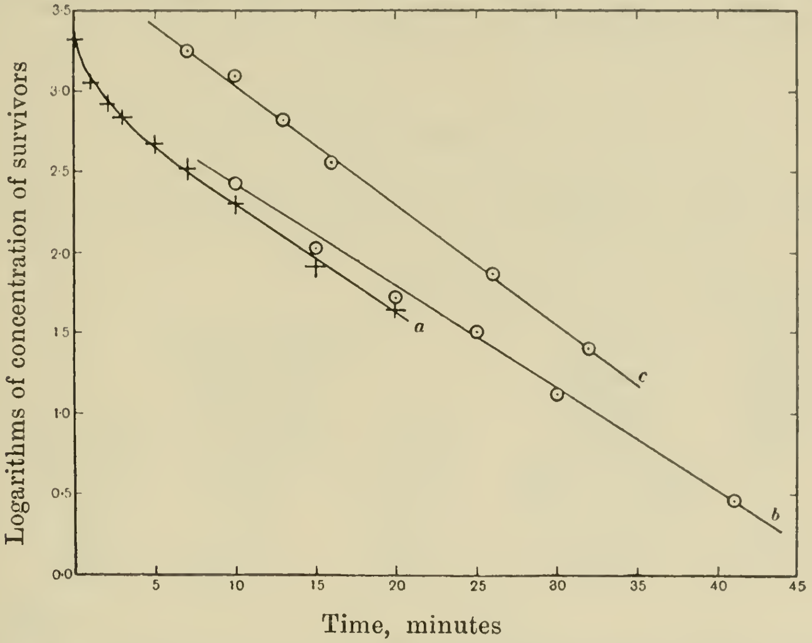

Fig. 12. Disinfection of *B. paratyphosus* with hot water (Table XIV).
(*a*) Exp. I at 47·2° C. (*b*) Exp. II at 47·2° C. (*c*) Exp. III at 47·35° C.

(*d*) *Staphylococcus pyogenes albus and Staphylococcus pyogenes aureus.*

Staphylococcus pyogenes albus. The results of two experiments with *Staphylococcus albus* at 49° C. and 53·05° C. respectively are given in Table XV and Fig. 13; they show fair agreement with the logarithmic law.

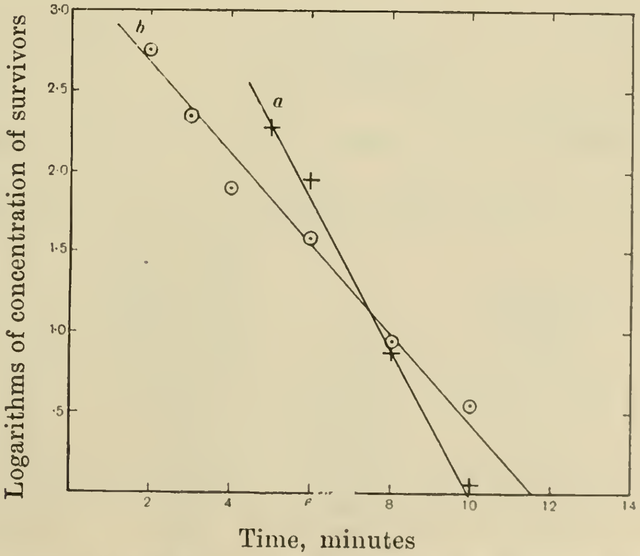

Fig. 13. Disinfection of *Staphylococcus pyogenes albus* with hot water (Table XV).
(*a*) Exp. I at 53·05° C. (*b*) Exp. II. at 49° C.

From analogy with other species one would expect the rate of disinfection at 53° C. (Exp. I) to be greatly in excess of that at 49° C. (Exp. II). The experiments were not made at the same time, and it must be concluded that this form, like *Staphylococcus pyogenes aureus*, is very variable as regards its resistance to disinfection by hot water.

TABLE XV.

Disinfection of Staphylococcus pyogenes albus with hot water.

Exp. I, 10. 3. '09. Temperature 53·05° C.

Time, minutes $= t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $= N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$5 = t_0$	2	371 393 376	380	$190 = N_0$	2·279	—
6	5	386 501 459	449	89·8	1·953	·33
8	10	60 88	74	7·4	·869	·47
10	10	9 14	11·5	1·15	·061	·44
					Mean	·41

Exp. II, 12. 3. '09. Temperature 49° C.

Time, minutes $= t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $= N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$2 = t_0$	1	575 513 599	562	$562 = N_0$	2·750	—
3	1	212 224 229	222	222	2·346	·40
4	1	96 51 88	78·3	78·3	1·894	·43
6	1	10 68	39	39	1·591	·29
8	5	20 82 30	44	8·8	·944	·30
10	10	39 31	35	3·5	·544	·28
					Mean	·34

TABLE XVI.

Disinfection of Staphylococcus pyogenes aureus with hot water at 49·3° C.

Exp. 21. 5. '09. (24 hours' culture from stock culture 44 days old.)

Time, minutes $= t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $= N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$1 = t_0$	1	1826 1904	1865	$1865 = N_0$	3·271	—
2	1	1457	1457	1457	3·163	·108
3	1	929 1279	1104	1104	3·043	·114
7	1	517	517	517	2·713	·093
10	1	416 443	429	429	2·632	·071
15	2	283	283	141·5	2·149	·080
					Mean	·093

Staphylococcus pyogenes aureus. On examination of the results of experiments with *Staphylococcus pyogenes aureus* (Tables XVI to XIX and Figs. 14 to 17), it is seen that in this case disinfection by hot water conforms to no constant type. In almost all cases the latter part of the disinfection proceeds logarithmically (see Exps. I and II, Table XIX, and Fig. 17, which are concerned with the course of disinfection after 10 minutes and 16 minutes respectively have elapsed), but the time relations of the disinfection process as a whole present great variety among which three distinct types can be distinguished:

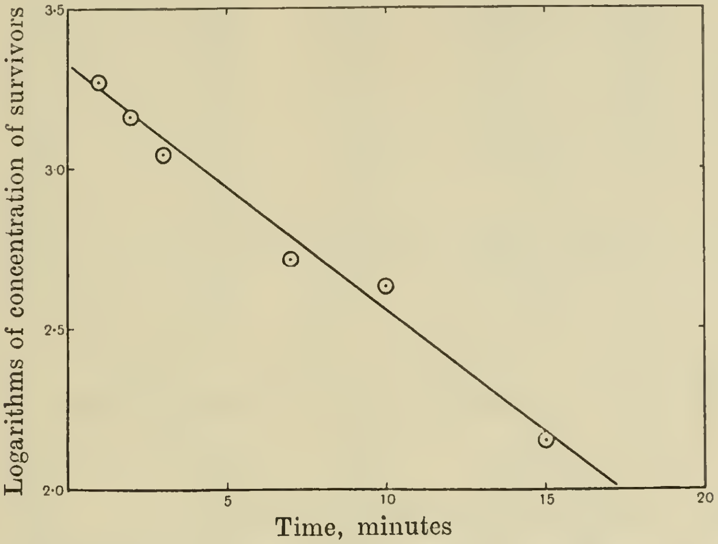

Fig. 14. Disinfection of *Staphylococcus pyogenes aureus* with hot water at 49·3° C. (Table XVI).

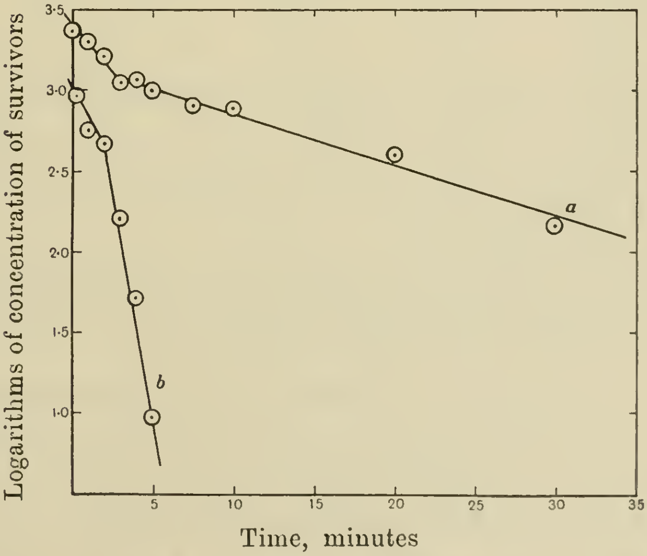

Fig. 15. Disinfection of *Staphylococcus pyogenes aureus* with hot water (Table XVII).
(*a*) Exp. I at 49·3° C. (*b*) Exp. II at 49° C.

TABLE XVII.

Disinfection of Staphylococcus pyogenes aureus with hot water.

Exp. I, 13. 5. '09. (24 hours' culture from stock culture 36 days old.) Temp. 49·3° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$1 = t_0$	1	2168 2522 2024	2238	$2238 = N_0$	3·350	—
2	1	1698 1649 1516	1621	1621	3·210	·140
3	1	1105 1182 1119	1135	1135	3·055	·147
4	1	1068 1343 1125	1179	1179	3·072	·093
5	1	971 1023 1082	1025	1025	3·011	·085
7	1	819	819	819	2·913	·073
10	2	1372 1558 1619	1516	758	2·880	·052
20	3	1320 1160	1240	413	2·616	·039
30	7 10	1101 1388	— —	146	2·164	·041
Exp. II, 3. 6. '09. (24 hours' culture from stock culture 13 days old.) Temp. 49° C.						
$0·25 = t_0$	1	912	912	$912 = N_0$	2·960	—
1·0	1	525 570	547	547	2·738	·30
2	1	542 393	467	467	2·669	·17
3	1	224 108	166	166	2·220	·27
4	1	42 60	51	51	1·708	·33
5	2	22 14 20	18·66	9·33	·970	·42
					Mean	·30

(1) Disinfection rate is throughout approximately proportional to the concentration of survivors (Table XVI), and a straight line best expresses the relation of the points when logarithms of survivors are plotted against time (Fig. 14).

(2) There is a "period of lag" at the beginning of disinfection (see Exp. II, Table XVII, and Exps. I, II and III, Table XVIII, where the value of K progressively increases) recalling disinfection of this species with phenol; a convex curve is obtained when logarithms of concentration of survivors are plotted against time (Fig. 15 *b* and Fig. 16).

(3) Disinfection is much accelerated at the beginning and becomes slower afterwards (see Exp. I, Table XVII, where the value of K progressively decreases); a concave curve is obtained when logarithms of concentration of survivors are plotted against time (see Fig. 15 *a*).

TABLE XVIII.

Disinfection of Staphylococcus pyogenes aureus by hot water.

(24 hours' culture from very young stock cultures.)

Exp. I, 24. 1. '10. (24 hours' culture from stock culture 5 days old.) Temp. 48·9° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (gelatine)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$		$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$
$0·25=t_0$	1	1800 1790 1090	1560	$1560=N_0$	3·193		—
2·0	1	1230 1330	1280	1280	3·107	Mean ·0486	·0491
5	2	1890 1930 2010	1940	970	2·987		·0434
10	2	980 910	945	472·5	2·674		·0532
20	7 10	29 52	— —	4·8	·681		·127

Exp. II, 24. 1. '10. (24 hours' culture from stock culture 6 days old.) Temp. 52·9° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (gelatine)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$		$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$
$0·25=t_0$	1	1230 1110 1180	1170	$1170=N_0$	3·068		—
1·0	1	990 840 810	880	880	2·944	Mean ·188	·165
2	2	1020 1270	1145	572	2·757		·176
5	6 10	590 1130 940	— —	102	2·009		·223
10	10	7 0 0	2·3	0·23	– ·638		·380

Exp. III, 9. 2. '10. (24 hours' culture from stock culture 2 days old.) Temp. 52·6° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (gelatine)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$		$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$
0·25	1	Lost					
$1·0=t_0$	1	517 594	555·5	$555·5=N_0$	2·745		—
2	1	353	353	353	2·548	Mean ·223	·197
4	2	264 195	229·5	114·7	2·060		·228
6	5	182 151 176	169·7	33·9	1·530		·243
8	8 10	29 26	— —	3·1	·491		·322
10	20	4	4	0·2	– ·699		·344

The reason[1] for this irregularity was not discovered, it is possible that the cause lies in the variability of the species and may depend upon the special character of the stock culture from which the 24 hours' culture was prepared. It was generally true that disinfection of cultures made from fresh stock cultures (under a week old) was of the 2nd type, while that of material obtained from older stock cultures conformed to the 1st or 3rd type.

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[1] The suggestion is made that in some cases these irregularities are only apparent, and may be due to agglutinations, taking place at once (type 3), or after an interval of time (type 2), when an emulsion of the bacteria is suspended in hot water. Cultures of Staphylococcus readily agglutinate when suspensions in water or broth are heated to temperatures somewhat higher than those employed in the present experiments. Attempts were therefore made to trace microscopically any agglutination that might occur, but nothing significant could be detected. It is however certain that an agglutination which would apparently increase the rate of disinfection three or four times (see Tables XVII and XVIII) would be very insignificant and almost impossible to detect with the microscope.

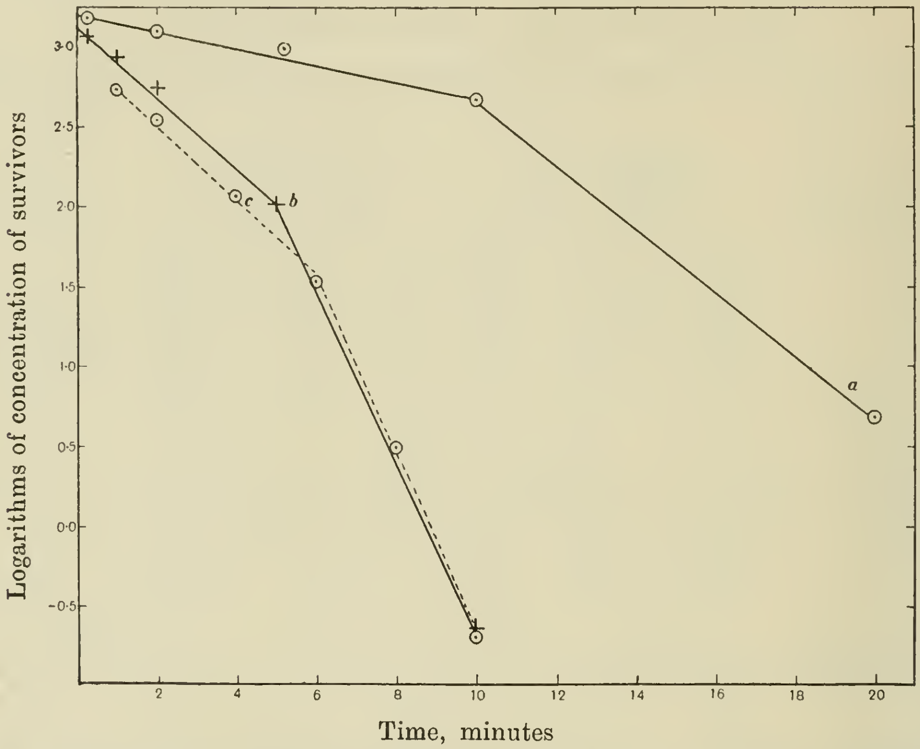

Fig. 16. Disinfection of *Staphylococcus pyogenes aureus* with hot water (Table XVIII). (*a*) Exp. I at 48·9° C. (⊙). (*b*) Exp. II at 52·9° C. (+). (*c*) Exp. III at 52·6° C. (⊙).

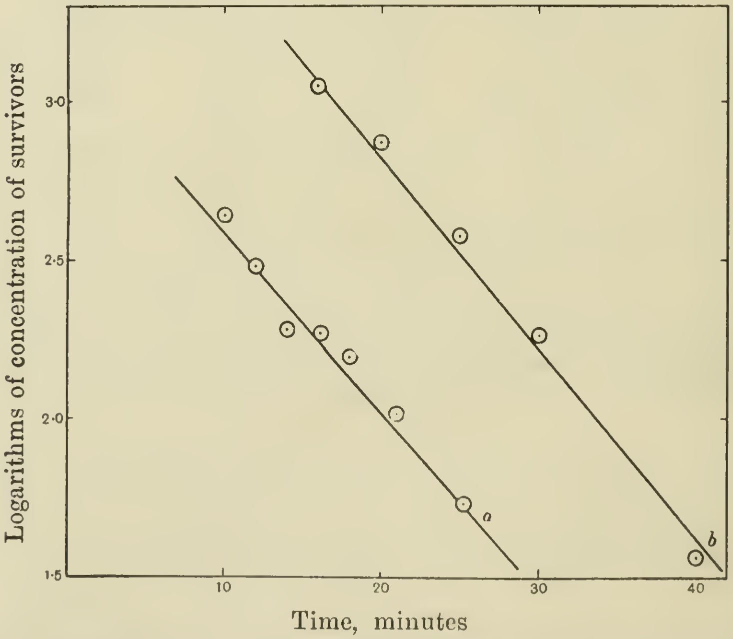

Fig. 17. Disinfection of *Staphylococcus pyogenes aureus* by hot water at 49·3° C. (Table XIX).
(*a*) Exp. I. (*b*) Exp. II.

TABLE XIX.

Disinfection of Staphylococcus pyogenes aureus with hot water at 49·3° C.

Exp. I, 8. 4. '09. (24 hours' culture from stock culture 11 days old.)

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$
$10=t_0$	1	458 372 489	439·7	$439{\cdot}7=N_0$	2·643	—
12	1	332 271 306	303	303	2·481	·081
14	2	441 369 341	384	192	2·283	·090
16·1	3	555 527 598	560	187	2·272	·061
18	5	821 788 755	788	158	2·199	·055
21	4 5 10	409 598 608 972	— — —	107·8	2·033	·055
25·25	10	450 488 666	535	53·5	1·728	·060
					Mean	·067

Exp. II, 14. 4. '09. (24 hours' culture from stock culture 7 days old.)

$16=t_0$	1 2	1153 1243 2109	— —	$1126=N_0$	3·051	—
20	3	2158 2207 2352	2239	746	2·873	·044
25	5	1976 1846 1845	1889	378	2·577	·053
30	10	1818 1837 1803	1819	181·9	2·260	·056
40	10	305 412 373	363	36·3	1·560	·062
					Mean	·054

Besides a general want of consistency in the character of the disinfection process itself, the average velocity of disinfection under apparently similar conditions is also extremely variable. Great difficulty in obtaining constancy of resistance in cultures would appear to be characteristic of work with Staphylococcus. In disinfection by hot water it was unusual to obtain the same result twice, unlooked-for alterations in resistance were constantly occurring, for which the responsibility was not satisfactorily traced. For example, Table XVII, Experiment II, shows a value of the velocity constant which is nearly three times that of the average in Experiment I, and four to five times that in Experiments I and II, Table XIX, all of which were made under almost identical conditions as regards temperature.

There is no entirely satisfactory explanation for these inconsistencies. As mentioned above, I find that the previous history of cultures of Staphylococcus modifies very considerably their behaviour towards disinfectants; in the case of disinfection with phenol, difference in temperature of incubation had an unexpectedly great influence upon the resistance of the bacteria towards phenol (see above, p. 247). It is probable that it also modifies its resistance to hot water.

(*e*) *B. pestis.*

The results of one experiment with *B. pestis* at 50° C. (Table XX and Fig. 18) show fair agreement with the logarithmic law.

TABLE XX.

Disinfection of B. pestis with hot water at 50° C.

Time, minutes $=t$	No. of organisms in 5 drops disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$4 \cdot 25 = t_0$	$9440 = N_0$	3·975	—
7·0	6880	3·838	·050
9·75	6160	3·790	·034
13·0	4460	3·649	·037
16·0	3340	3·524	·038
		Mean	·040

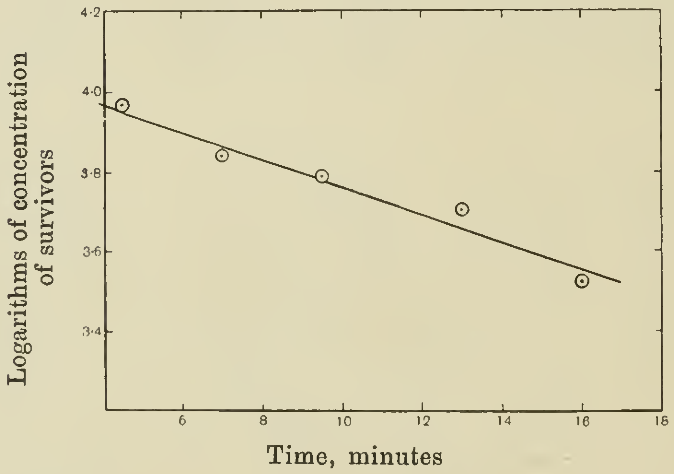

Fig. 18. Disinfection of *B. pestis* with hot water at 50° C. (Table XX).

(3) *Temperature coefficient of disinfection by hot water.*

B. typhosus. The experiments with *B. typhosus* extended over a range of 5° C., and the temperature coefficient of disinfection by hot water could therefore be determined with some accuracy by comparing velocity constants of two comparable disinfections at different temperatures. In Experiments I and II, Table X, the mean value of the velocity constant at 54·1° C. was 1·45, while that corresponding to 49° C. was 0·111. This gives an increase in reaction velocity of 13·1 times for 5° C. rise in temperature, *i.e.* 1·67 times per 1° C., and about 170 times per 10° C. rise in temperature, if the temperature effect is assumed to be consistent.

To ascertain the truth or otherwise of this assumption and for purposes of comparison, some additional experiments were made with *B. typhosus*, using an end-point method (see H. C. 1908, p. 118). Test tubes containing 5 c.c. of distilled water and fitted with standardised capillary pipettes were placed in thermostats maintained at different temperatures and sown with a small quantity (5 standard drops = 0·1 c.c.) from a 24 hours' culture of *B. typhosus*. From time to time samples (4 drops = 0·08 c.c.) were withdrawn by means of the capillary pipette and added to tubes of glucose broth, which were then incubated. The time necessary for complete disinfection was taken as the mean between that of the last positive and first negative test culture. These times of "complete disinfection" strictly mean the time taken for an initial concentration of about 5,000,000 bacteria per c.c. to be reduced to less than 12 per c.c., *i.e.* till there may not be one living bacteria present in the sample taken, 0·08 c.c.

The results of two experiments (Table XXI) are consistent one with the other. A logarithmic relation was found to express the effect of temperature upon disinfection velocity, as was expected from analogy with other cases of disinfection which have been investigated. Logarithms of times taken for disinfection (reciprocals of mean reaction velocity) were found to be proportional to the differences of temperature, and, when plotted one against the other, gave approximately a straight line, see Fig. 19.

Whether rise of temperature affects the velocity of disinfection by hot water in a simple logarithmic manner or in accordance with the Law of Arrhenius, it is impossible to say, for within a small range of temperature the two become almost identical. The somewhat rough experimental method could not be expected to give very strict mathematical agreement with either, though as a matter of fact satisfactory agreement with both is obtained (see also temperature coefficient of disinfection with disinfectants, H. C. 1908, p. 153).

In any case the effect of temperature upon disinfection with hot water is seen to be consistent and very great. From Table XXI the mean value 1·60 is obtained as temperature coefficient per 1° C. From the slope of the smoothed curve in Fig. 19 it is seen that a rise of 10° C. in temperature from 49° C. to 59° C. indicates an increase of 2·00 in the logarithms of the times taken, *i.e.* an increase in the mean velocity of disinfection of 100 times, or 1·58 times for 1° C. These values are in fair agreement with that obtained by comparing velocity constants, viz. 1·67 per 1° C.

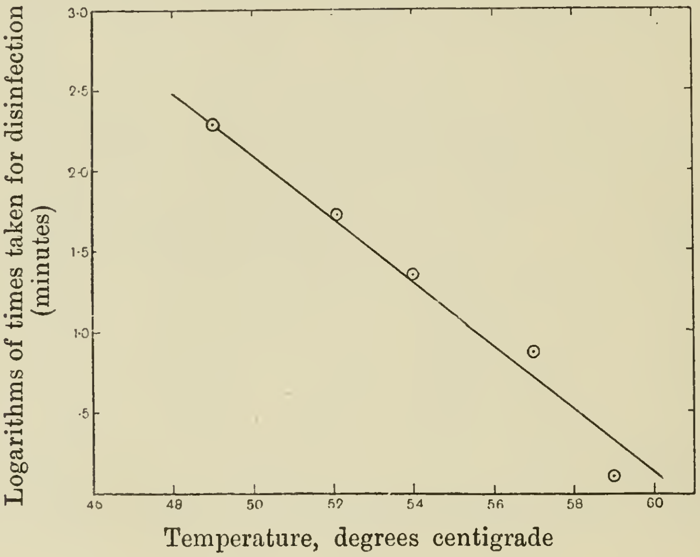

Fig. 19. Effect of different temperatures upon disinfection of *B. typhosus* by hot water (Table XXI).

TABLE XXI.

Temperature coefficient of disinfection of B. typhosus with hot water (end-point method).

	Temp. of exp., ° C.	Time taken for disinfection*, minutes $= t$	Log$_{10}$ t	Log$_{10}$ (temperature coefficient for 1° C.)	Temperature coefficient per 1° C.
Exp. I, 10. 6. '09	59	1·26	·100	·250	1·78
	54	22·5	1·352	·188	1·54
	49	196	2·292		
Exp. II, 4. 6. '09	57	7·5	·875	·173	1·46
	52·1	53	1·724		
				Mean	1·60

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* Strictly speaking, reduction of about 5,000,000 per c.c. to less than about 12 per c.c.

B. coli commune and Staphylococcus pyogenes aureus.

In the preceding experiments with *B. coli commune* and *Staph. py. aur.* there are a few sets which can be used for calculation of the temperature coefficient of disinfection by hot water, assuming the effect of temperature to be consistent and in accordance with a logarithmic law. These are collected in Table XXII. Both species are extremely variable, and it is necessary, even more than was the case with *B. typhosus*, that for comparison of disinfection rate at different temperatures the experiments should be carried out on the same day, with the same material, etc. In Table XXII, for example, it will be seen that whereas on two occasions velocity constants obtained for the disinfection of *B. coli* at practically the same temperatures are very different, the temperature coefficients reckoned from them are very similar, 1·23 and 1·32 per 1° C.

TABLE XXII.

Temperature coefficient of disinfection by hot water, B. coli commune and Staphylococcus pyogenes aureus.

	Organism	Temp., ° C.	Difference in temp., ° C.	Mean value of velocity constant, K	Temp. coefficient per 1° C.
Exp. 15. 2. '10	*B. coli commune*	48·9	3·8	·172	1·23
(Table XII)	,,	52·7		·381	
Exp. 26. 2. '10	*B. coli commune*	49	3·5	·0424	1·32
(Table XIII)	,,	52·5		·111	
Exp. 24. 1. '10	*Staph. py. aur.*	48·9	4·0	·0486	1·40
(Table XVIII)	,,	52·9		·188	

The temperature coefficient of disinfection with hot water considerably exceeds that of disinfection by other means. Disinfection of *B. paratyphosus* with phenol and other coal-tar disinfectants was found to have a temperature coefficient of 10 per 10° C. under certain conditions, while with metallic salts the figure was very much lower and approximated to that usually obtaining in chemical reactions. Disinfection by drying has also a low temperature coefficient, viz. 2 to 3 for 10° C. (Paul, 1909 and Paul, Birstein and Reuss, 1910). In the case of disinfection by hot water we have figures ranging from about 136 per 10° C. (1·635 per 1° C., if we take the mean of the values obtained by the two different methods) in case of *B. typhosus*, to 29 per 10° C. (1·40 per 1° C.) in case of *Staphylococcus* and 12 per 10° C. (1·28 per 1° C.) in case of *B. coli*.

The figures in cases like that of *B. coli* and *Staph. py. aur.*, where data are scanty, must only be considered approximate. The high temperature coefficient makes experiment impossible except over a very small range of temperature, and one can only obtain the effect for 10° C. by calculation; a small error, therefore, in the value obtained for 1° C. will lead to a large one when this value is raised to the 10th power to obtain the value for 10° C. rise in temperature.

(4) *Influence of acids and alkalis.*

The influence of reaction upon disinfection by hot water was noticed in the course of some experiments in which a suspension of *B. typhosus* in broth was the material used for investigation. The experiments were made in order to obtain information which might be of service in the manufacture of vaccines. The temperature was 54° C. The method of experiment was exactly as in the preceding experiments, except that, in place of distilled water, the bacteria were suspended in broth in which the same species had grown for 40 hours at 37° C. and which had been sterilised by filtration through a Berkefeld filter.

Disinfection was logarithmic, but the rate was much slower than in distilled water under the same conditions. This was found to be due to the slight alkalinity of the broth. On addition of a small quantity of acid (0·25 c.c. $\frac{N}{1}$ acetic acid to 20 c.c. broth) up to neutralisation point (litmus), the rate of disinfection was increased about 8 times, see Table XXIII, Experiments of 22. 7. '09 and Fig. 20. A small extra amount of acid, too small to exert any disinfectant action of itself[1] (in all 0·5 c.c. $\frac{N}{1}$ acetic acid added to 20 c.c. broth), rendered disinfection so quick that its rate could not be measured, see Table XXIV, Experiment II. In this case, the value of the velocity constant of disinfection

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[1] Acetic acid in the presence of 1 % peptone has been shown to exert a disinfectant action upon *B. coli commune* at and above a concentration of $\frac{N}{11}$ (Winslow and Lockridge, 1906). *B. typhosus* is probably more susceptible, but in the above case (0·25 c.c. $\frac{N}{1}$ acetic acid to 20·25 c.c. neutralised broth) it is likely that the acidity of the medium fell far short of $\frac{N}{100}$. The broth contained besides 1 % peptone, a considerable proportion of meat extractives, which also, by combining with the acid added, would tend to further reduce the concentration of free acid in the medium.

TABLE XXIII.

Effect of small differences in reaction upon the disinfection of B. typhosus in broth at 54·1° C.

EXP. I, 22. 7. '09. Medium = filtered culture (40 hrs. at 37° C.) of *B. typhosus*.

Time, minutes $=t$	Amount of sample plated, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$0{\cdot}32 = t_0$	1	2796 2888 2954	2879	$2879 = N_0$	3·459	—
1·07	1	1704 1982 1894	1860	1860	3·270	·25
2·15	2	1160 1029 1187	1125	562	2·750	·39
3·50	3	565 573 504	547·3	182·4	2·261	·38
6·00	5	157	—	38·1	1·581	·33
	9	339	—			
	10	418	—			
10·00	10	20 20	—	1·62	·209	·34
	20	25	—			
					Mean	·34

EXP. II, 22. 7. '09. Medium = neutralised filtered culture (40 hrs. at 37° C.) of *B. typhosus* (10 drops = 0·25 c.c. $\frac{N}{1}$ acetic acid added to 20 c.c. broth).

Time, minutes $=t$	Amount of sample plated, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$0{\cdot}28 = t_0$	1	590 602 679	624	$624 = N_0$	2·795	—
0·53	1	154 36 266	152	152	2·182	2·45
1·0	3	40 19 40	33	11	1·041	2·44
1·5	3	2 0 0	0·66	0·22	– ·658	2·83
					Mean	2·57

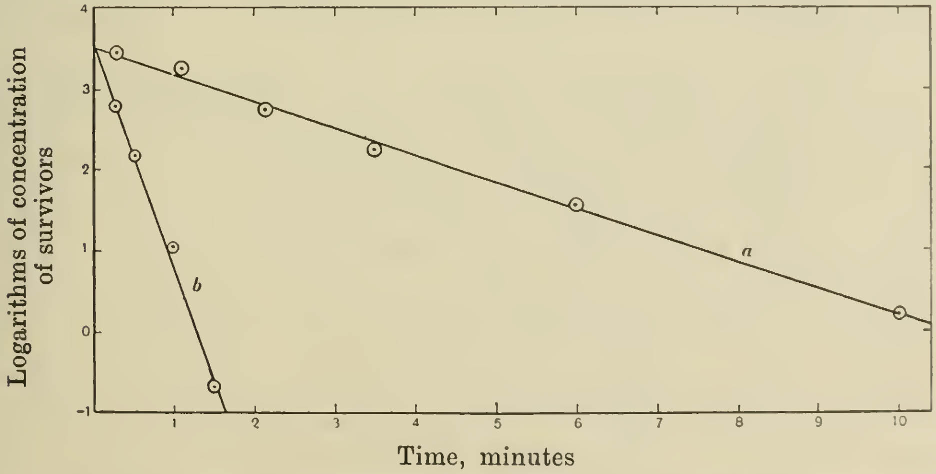

Fig. 20. Effect of reaction upon disinfection of *B. typhosus* in broth at 54·1° C. (Table XXIII).

(*a*) Exp. I, broth slightly alkaline.

(*b*) Exp. II, broth made just neutral (litmus) with acetic acid.

in the original broth was 0·44; in the faintly acid broth, where about 3000 organisms per drop were reduced to less than 1 per drop in 15 seconds, the value of the velocity constant was more than 17·6, *i.e.* more than 40 times as great as in the original.

TABLE XXIV.

Effect of small differences in reaction upon the disinfection of B. typhosus in broth at 54° C.

Exp. I, 24. 7. '09. Medium = filtered culture (40 hrs. at 37° C.) *B. typhosus.*

Time, minutes $=t$	Amount of sample plated, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$0·27 = t_0$	1	2640 2816 3232	2896	$2896 = N_0$	3·462	—
0·98	1	2068 1420 1754	1747	1747	3·242	·31
2·00	1	165 240 325	243	243	2·386	·62
3·53	3	357 351	354	118	2·072	·43
6·17	5	59 40	49·5	9·9	·996	·42
10·27	10	1 3	—	0·125	−·903	·44
	20	1	—			
					Mean	·44

Exp. II, 24. 7. '09. Medium = filtered culture (40 hrs. at 37° C.) *B. typhosus* + 20 drops (0·5 c.c.) $\frac{N}{1}$ acetic acid to 20 c.c. broth.

Time, minutes $=t$	Amount of sample plated, drops	Numbers counted on plates (agar)	Mean	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
$0·25 = t_0$	1	0 0 0	—	—	—	More than
0·67	3	0 0 1	—	—	—	17·6*

* In Exp. II, disinfection after 0·25 minutes is more complete than after 10·27 minutes in Exp. I, *i.e.* disinfection rate is more than 40 times that of Exp. I, K = more than 17·6.

For the sake of comparison with the above experiments were made to investigate the influence of reaction upon disinfection in distilled water. In Tables XXV and XXVI are set forth the results of two comparable sets of experiments, in which *B. typhosus* was heated at 54° C. in water to which were added various small amounts of acid[1] and alkali insufficient in themselves to exert a disinfectant action. The smallest amounts which were employed were sufficient to render the solutions acid or alkaline to the extent of $\frac{1}{7000}$th N respectively. In the case in which alkali was added, the solution was just alkaline to phenol phthalein. These small amounts were not without effect upon the rate of disinfection by hot water, which in the case of alkali was increased about 1·5 to 2·0 times, and in the case of acid 5 to 7 times. On further addition of acid disinfection became too quick to study, whereas the increase in rate on adding more alkali was comparatively slight.

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[1] Death rate at 20° C. of *B. typhosus* suspended in water containing a concentration of $H_2SO_4 = \frac{N}{500}$ was found to be insignificant. Disinfection by H_2SO_4 may be assumed to have a low temperature coefficient from analogy with other mineral disinfectants.

It therefore follows that, in the preparation of vaccines, no conclusion drawn from one set of experiments can be usefully applied to another set, unless the composition and reaction of the medium, in which the bacteria are heated, be kept strictly constant. This is almost impossible for, in the case of broth cultures, the reaction of the broth will, within small limits, depend upon the extent of the growth of organisms. It is not possible to control this, and small differences arising therefrom will have a very significant influence upon the rate of destruction by heat of the organisms suspended in the medium.

TABLE XXV.

Effect of small amount of acid and alkali upon disinfection of B. typhosus in distilled water at 54° C.

EXP. 4. 8. '09. Distilled water.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\mathrm{Log}_{10} N$	$K = \frac{1}{t_n - t_0} \log \frac{N_0}{N_n}$
0·23 = t_0	1	3360 3700	3530 = N_0	3·548	—
0·55	1	2260 2240 2660	2390	3·378	·531
1·07	3	2780 2640	903	2·956	·705
2·5	9 10	1400 2370 1970	198	2·297	·551
					Mean ·596
$\frac{N}{7000}$ alkaline (NaOH)*.					
0·25 = t_0	1	1220 1380	1300 = N_0	3·114	—
0·90	1	245 183 160	196	2·292	1·26
2·05	1 3	11 49 39	14·1	1·149	1·09
					Mean 1·17
$\frac{N}{7000}$ acid (H_2SO_4).					
0·22 = t_0	1 10	294 2490	253 = N_0	2·403	—
0·51	10 20	217 296	17·1	1·233	4·03
0·92	10 20	111 114	7·5	·875	2·18
					Mean 3·10

* Just alkaline to phenolphthalein.

TABLE XXVI.

Effect of small amount of acid and alkali upon disinfection of B. typhosus in distilled water at 52° C.

Time, minutes $=t$	Amount of sample taken, drops	Numbers counted on plates	Mean no. of bacteria present in 1 drop disinfection mixture, $=N$	$\text{Log}_{10} N$	$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$
Exp. I, 19. 8. '09. Distilled water.					
$0{\cdot}33=t_0$	1	7250 5420 5350	$6010=N_0$	3·779	1·65
1·0	3	7850 5500	2225	3·347	
Exp. II. $\frac{N}{7000}$ **alkaline (NaOH)*.**					
$0{\cdot}33=t_0$	2	6930 6230 7550	$3450=N_0$	3·538	·95
1·0	15	11340	805	2·906	
	20	15260 17660			
Exp. III. $\frac{N}{3500}$ **alkaline (NaOH).**					
$0{\cdot}33=t_0$	5	3840 3780 3670	$753=N_0$	2·877	1·26
1·0	16	1910	109	2·037	
	20	2020			
Exp. IV. $\frac{N}{1750}$ **alkaline (NaOH).**					
$0{\cdot}33=t_0$	10	6180 7860 8780	$761=N_0$	2·881	1·26
1·0	19	1780	111	2·045	
	20	2540			
Exp. V. $\frac{N}{875}$ **alkaline (NaOH).**					
$0{\cdot}33=t_0$	16	9250	$587=N_0$	2·769	1·31
	20	11870			
1·0	19	1600	78·5	1·895	
	20	1460			
Exp. VI. Distilled water.					
$0{\cdot}33=t_0$	1	930 1020 1130	$1030=N_0$	3·013	·62
1·0	3	760 1280	393	2·594	
	5	2280			
Exp. VII. $\frac{N}{7000}$ **acid** (H_2SO_4).					
$0{\cdot}33=t_0$	5	179 179	$35{\cdot}8=N_0$	1·554	at least 4·29
1·0	20	(?) 2 0	(?) 0·05	(?) −1·301	

* Just alkaline to phenolphthalein.

(5) *Analogy between the disinfection of bacteria by hot water and the "heat coagulation" of proteins.*

The disinfection of bacteria by heat in presence of water exhibits a striking analogy with the behaviour of some proteins under similar conditions, and leads to the inference that disinfection of bacteria by this means is due to hydration of their constituent proteins.

The suggestion has already been made that the difference in resistance to heat of vegetative and sporing forms is caused by the difference in the readiness with which their respective proteins are coagulated (Lewith, 1890, Hewlett, *loc. cit.* p. 20). Lewith (p. 349) adduces some experimental support for the view that this difference is to be attributed to a diminution in the water-content of spores. That coagulation is not a pure temperature effect is clear from the comparative security of both bacterial spores and proteins at high temperatures in the absence of water.

The analogy takes a more definite shape when the foregoing experiments with bacteria are compared with some recent work on the alteration of proteins by hot water.

Famulener and Madsen (1908) found that the destruction of three antigens when heated in the presence of water proceeded logarithmically and that the process had a very high temperature coefficient, being about 2 for a rise in temperature of 1° C. A similar set of results have been obtained by Madsen and Streng (1909) for a series of agglutinins.

The "heat coagulation of" two pure crystallised proteins, haemoglobin and egg-albumen, has recently been shown (Chick and Martin, 1910) to be an orderly time-process, the rate of which varies with alteration of temperature, reaction and other conditions. In the case of haemoglobin coagulation takes place logarithmically, the coagulation rate at any moment being proportional to the concentration of uncoagulated protein. In the case of crystallised egg-albumen the reaction is of a higher order, as the crystals consist of a series of salts of protein with the acid used in their preparation, which salts coagulate at different rates. The effect of temperature upon the rate of reaction was found to be unusually great, the temperature coefficient in the case of haemoglobin being 1·3 per degree centigrade, and, in the case of egg-albumen, 1·91 per degree centigrade.

These observations on proteins show that it is improper to speak of a protein as having a definite "coagulation temperature"; for the same reason, it is no longer permissible to talk of the "thermal death-point" of any particular species of bacteria. It is in both cases the possession of exceedingly high temperature coefficients which have rendered observation of these supposed constants of any practical value. This will be clear from some such example as the following. In the case of disinfection of *B. typhosus* with hot water, the temperature coefficient has been determined to be 1·635 for 1° C. rise in temperature. From the value of velocity constant in Table X, 0·111, it can be calculated that a suspension of *B. typhosus*, containing 100,000 bacteria per c.c., would be disinfected (the number reduced to less than 1, an average of 0·5, per c.c.) in 48 minutes at 49° C. From the above value of the temperature coefficient it can be calculated that the disinfection would take about 2 hours at 47°, 18 minutes at 51° C., 7 minutes at 53°, 2½ minutes at 55° and 21 seconds at 59°. If, therefore, a suspension of *B. typhosus* in water were gradually heated up, death would apparently take place suddenly at a temperature near 55° C., and it is easy to understand how some such temperature should come to be regarded as the "thermal death-point" for this species.

Another point of analogy between the "heat coagulation" of proteins and disinfection of bacteria by hot water is that both processes are similarly affected by the presence of minute quantities of acid, the rate being greatly increased in each case.

The striking similarity between the effects of temperature (dry) on the one hand, and hot water on the other indicate that disinfection by the latter is due to the action of water (coagulation or alteration) upon some one protein which is essential for the life of the bacterium, and that the character of the reaction is conditioned by the chemical action of water upon its constituent proteins.

(6) *Summary of Section III.*

1. (*a*) In the case of *B. typhosus*, *B. coli commune*, and *B. pestis* disinfection with hot water also proceeds according to a logarithmic law, the rate of disinfection at any moment being proportional to the concentration of surviving bacteria.

(*b*) In the case of *B. paratyphosus* the rate of disinfection at the beginning of the experiments was in excess of the theoretical, but in the course of a few minutes slowed down to a consistent rate. The same phenomenon had previously been observed in disinfection of this species with phenol and other disinfectants.

(*c*) In the case of *Staphylococcus* there was the greatest variability in the character of the disinfection process, which in some cases conformed to the ideal type (anthrax spores or *B. typhosus*), in others to that of *B. paratyphosus*, while in others again there was a period of "lag" at the beginning of disinfection recalling disinfection of *Staphylococcus* with phenol. Further complications were introduced by the lack of constancy in resistance to disinfecting agents which is characteristic of this species.

2. The effect of difference in temperature upon rate of disinfection by hot water has been shown to be consistent throughout the small range investigated and in accordance with the law of Arrhenius, or some similar logarithmic law. The temperature coefficient has been determined in some cases, and in that of *B. typhosus* reaches the high figure of 1·635 per 1° C., or 136 per 10° C. In the case of *B. coli commune* and *Staphylococcus pyogenes aureus*, the temperature coefficient was not studied with the same completeness but, from the few observations made, appears to be 1·28 and 1·4 respectively per degree centigrade.

The high temperature coefficient is comparable to that obtained for precipitation of proteins with hot water, and supports the view that disinfection of bacteria by this means is due to "heat coagulation" of their constituent proteins.

3. The presence of minute quantities of acid or alkali too small to produce any direct disinfectant action has a very marked influence upon disinfection by hot water. In both cases the rate of disinfection is increased, but in the case of acid to a much greater extent. Whereas, in the case of *B. typhosus*, the addition to distilled water of sufficient alkali to render the solution $\frac{N}{7000}$ alkaline increased the mean rate of disinfection at 54° C. about 1·5- to 2-fold, a similar addition of acid increased it 5- to 7-fold. Further addition of alkali influenced the disinfection-rate comparatively little; with further addition of acid it became too rapid for study. This presents a close analogy with the influence of small quantities of acid upon "heat coagulation" of proteins.

4. The effect of reaction was of the same order when suspensions of bacteria in broth were substituted for those in water. For this reason it is impossible to obtain standard conditions in the manufacture of vaccines, as owing to the growth of the bacteria it is impossible to control the reaction of the broth.

IV. DISINFECTANT ACTION OF SUNLIGHT AND DRYING.

Sunlight. The only suitable data upon this subject, which are available, are the results of some experiments by Clark and Gage (1903) in which the disinfectant action of sunlight was quantitatively studied upon *B. typhosus* and *B. coli commune* suspended in water. Most of the experiments give irregular results from which no conclusion can be drawn. In Table XXVII, however, are set forth the results of one experiment with *B. coli* which gives consistent results and which well repays analysis. The concentration of survivors, determined from time to time

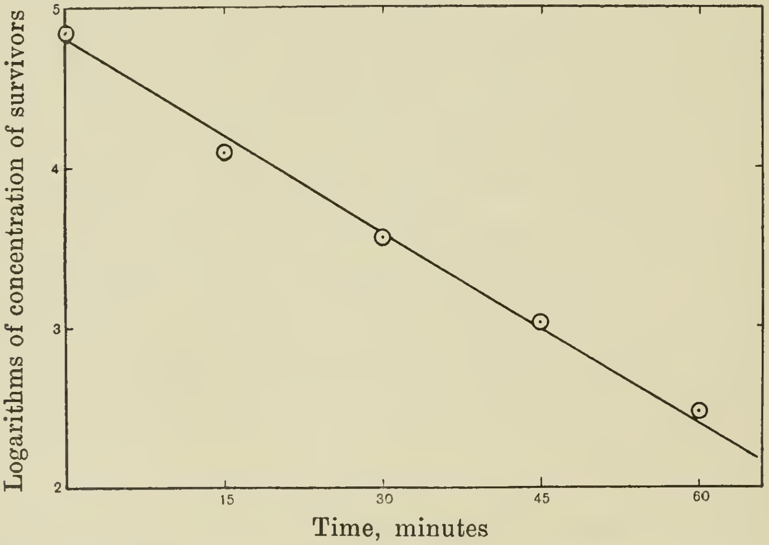

Fig. 21. Disinfection of *B. coli commune* by sunlight, results of Clark and Gage (1903), Table XXVII.

TABLE XXVII.

Disinfectant action of sunlight upon B. coli commune.

(Clark and Gage, *Thirty-fourth Annual Report, State Board of Health, Massachusetts*, 1903.)

Time, minutes $=t$	Concentration of surviving bacteria, numbers per c.c. $=N$	$\text{Log}_{10}\,C$	$K=\frac{1}{t_n-t_0}\log\frac{N_0}{N_n}$
$0=t_0$	$71400=N_0$	4·854	—
15	12365	4·092	·051
30	3568	3·566	·043
45	1065	3·027	·041
60	295	2·470	·040
		Mean	·044

by means of plate cultures, is seen to vary logarithmically with time, the value of the velocity constant, K, remains fairly constant in value, and a straight line is obtained when logarithms of concentration of survivors are plotted against time, see Fig. 21.

It is not justifiable to draw a definite conclusion from such scanty data, but it is more than probable, judging from analogy, that the process of disinfection by sunlight will be found to fall in line with that experimentally established for other agents.

Drying. In a preliminary examination of the suitability of the garnet[1] method for investigating the action of disinfectants upon *Staphylococcus*, Paul and Prall (1907) allowed garnets, coated with a thin dry film of this species, to remain at various temperatures and counted the surviving bacteria from time to time. At the temperature of liquid air the number of living bacteria remained roughly constant for many weeks, at ordinary temperature the number slowly decreased. Paul (1909) has recently analysed these data and shown that death—in this instance by drying—occurs in accordance with a logarithmic law and that the temperature coefficient for the process is about 2—3 for 10° C. rise in temperature (see also Paul, Birstein and Reuss, 1910).

V. SUMMARY AND GENERAL CONCLUSIONS.

1. The additional experimental evidence brought forward in the present paper lends further support to the view already put forward (1908) that disinfection is an orderly time-process, which may be considered analogous with a chemical reaction, viz. a reaction between the bacterium on the one hand, and the disinfectant on the other. In the ideal case disinfection proceeds in accordance with some rule analogous to the Mass Law, so that if the disinfectant is present in large excess, disinfection rate at any moment is proportional to the concentration of the bacteria $\left(-\frac{dn}{dt} = Kn\right.$, where n is the concentration of bacteria at the time t, and K is a constant depending on the temperature, concentration of disinfectant, etc.).

2. The above view was originally put forward on the basis of experiments with anthrax spores and *B. paratyphosus*, using a series of

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[1] Krönig and Paul (1897).

different disinfectants (H. C. 1908). In the present paper the results of further experiments with phenol are given in confirmation, viz. with *B. typhosus, B. coli commune* and *Staphylococcus pyogenes aureus.*

3. Disinfection with phenol and other disinfectants has already (*loc. cit.*) been shown to be influenced by temperature in accordance with the Law of Arrhenius. The effect upon disinfection rate of alteration in concentration of disinfectant (*loc. cit.* p. 117, and H. E. Watson, 1908) is also in accord with the view expressed in 1.

4. Further confirmation has been obtained from the case of destruction of bacteria by hot water between the temperatures of 45° C. and 55° C. Investigations on this subject are described in the present paper, and a very impressive parallel shown to exist between this case of disinfection and the heat coagulation of proteins. Both are consistent time-processes proceeding in uncomplicated cases in accordance with the Mass Law, influenced consistently by change of temperature in agreement with the Law of Arrhenius, and both possessing an extraordinarily high temperature coefficient. Both are similarly influenced by addition of minute quantities of acid.

The organisms worked with were *B. typhosus, B. coli commune, B. paratyphosus, Staphylococcus pyogenes aureus* and *B. pestis.*

5. Disinfection by drying (Paul, 1909) and, as far as can be judged from very scanty data (Clark and Gage, 1903), by sunlight fall into line with the other cases of disinfection which have been investigated.

The facts relating to the disinfection process have not been questioned, but some amount of criticism has been aroused by the explanation which has been suggested on the following lines.

As regards disinfection (whether by "disinfectants," hot water, sunlight, drying) a culture of bacteria consists of a uniform population. The fact that the individuals do not die all at once but at a rate proportional to the concentration of the survivors at a given moment is to be attributed to temporary and rhythmical changes in resistance which, by analogy with chemical processes, may be supposed to be due to temporary energy changes of the constituent proteins.

This simple law has been found to express the relation of mortality to time for the germicidal action of chemical disinfectants and heat upon anthrax spores and very closely, in my experiments, upon *B. typhosus, B. coli* and *Staphylococcus* (phenol). The deviation or departure from the law, most marked in the case of *B. paratyphosus*, has in my experience always been in one direction[1], viz. the rate of destruction falls more quickly than would be accounted for by the change in concentration of survivors alone. Here too, I believe the rate to be determined by concentration but to be complicated by the individual bacteria possessing more or less permanent differences in resistance.

Hewlett (*loc. cit.*) and Reichel (1909, p. 152) consider that the progressive nature of disinfection can be more naturally explained by supposing it to be due to differences in resistance occurring among the various bacteria[2]. If such were the case, disinfection would certainly be gradual and not sudden, but the rate would only be proportional to the concentration of survivors if the different resistances were allotted according to one definite arrangement, an arrangement which could only occur as a remote coincidence, and could hardly be universally present. For, suppose that the disinfection of 100,000 bacteria is in question, that the survivors vary logarithmically with time, as in the ideal case of disinfection, and that for the sake of argument the rate happens to be a reduction to $\frac{1}{10}$th in each minute,

$$10{,}000 = \frac{1}{10} \text{ of } 100{,}000, \text{ survive after 1 minute,}$$

$$1000 = \frac{1}{10} \text{ of } 10{,}000, \text{ will survive after 2 minutes, and}$$

$$100 = \frac{1}{10} \text{ of } 1000, \text{ will survive after 3 minutes,}$$

$$10 = \frac{1}{10} \text{ of } 100, \text{ will survive after 4 minutes, and so on.}$$

If the cause of this is to be sought in the possession by the bacteria of different individual resistances to disinfection we should have to suppose that these resistances had the following distribution among the 100,000 bacteria, viz. that

90,000 take 1 minute to kill,
9000 take 2 minutes to kill,
900 take 3 minutes to kill,
90 take 4 minutes to kill, and so on.

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[1] Cases in which departure from the logarithmic law is in the direction of preliminary lag may be explained by supposing it to be due to a delay in the disinfectant getting to work; after such delay disinfection usually proceeds in the ideal way, disinfection being proportional to the concentration of survivors (see disinfection of *Staphylococcus*, p. 244).

[2] A similar view was expressed by Bellei (1904).

Or, in the general case, if N bacteria die logarithmically, and if $\frac{1}{x}$ of N survive 1 minute, then

$$\frac{1}{x^2}N \text{ will survive 2 minutes,}$$

$$\frac{1}{x^3}N \text{ will survive 3 minutes, and so on,}$$

and the resistance of the various individuals will be so distributed that there are present

$$N-\frac{N}{x}, \text{ or } N\frac{(x-1)}{x}, \text{ which are killed in 1 minute,}$$

$$N\left(\frac{x^2-1}{x^2}-\frac{x-1}{x}\right), \text{ or } N\frac{(x-1)}{x^2}, \text{ which take 2 minutes to kill,}$$

$$N\left(\frac{x^3-1}{x^3}-\frac{x-1}{x^2}-\frac{x-1}{x}\right), \text{ or } N\frac{(x-1)}{x^3}, \text{ which take 3 minutes to kill,}$$

and no other distribution will satisfy the necessary condition.

A further argument can be brought against a theory of variable permanent resistance, viz. that a chance characteristic is as a rule distributed in a different manner. Those individuals possessing it in moderate amount are usually in the greatest number, while those possessing it in greater or less degree are in the minority. In the case of disinfection, those apparently possessing the least resistance are invariably in the greatest number.

A more rational explanation is based upon the essential similarity of the individual bacteria, for in no other way can the logarithmic nature of disinfection in the ideal case be reasonably accounted for. If one condition be necessary for death, or a multitude of small causes, among a number of similar individuals, the result to be governed by the law of probability, death will occur logarithmically (see Yule, 1910). In order to obtain a mental picture of the disinfection process, one is led to seek an analogy with other processes, which also occur logarithmically, viz. chemical reactions of the first order such as the decomposition of hydrogen arsenide, the inversion of sugar and, under certain conditions, the "heat coagulation" of proteins. The closest possible analogy has been shown to exist between the last instance and the destruction of bacteria by hot water. In these cases an explanation has been sought in temporary changes in the energy of the molecules, as a consequence of which all molecules do not possess the same sensibility to attack at the same moment. Some such property is therefore attributed to the molecules (or aggregates of molecules) of the constituent protein of the bacteria, whereby at a given moment only a certain proportion is liable to attack; the amount being dependent upon the concentration at the moment of unaltered protein, in other words the number of bacteria[1] surviving in unit volume.

Disinfection, whether by disinfectants or by heat, may be considered analogous to a chemical reaction the velocity of which is controlled by external conditions such as temperature or concentration of bacteria and disinfectant.

In conclusion, I gratefully acknowledge the great help I have received from Dr C. J. Martin, F.R.S., whose valuable advice and practical assistance have been at my disposal throughout this work. I am also indebted to him for the design of the very convenient apparatus, shown in Fig. 8, used for investigating disinfection by hot water.

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[1] Disinfection is ordinarily arranged so that the disinfectant is present in large excess, and its concentration may be considered to remain constant during the process.

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No 10

ON THE "HEAT COAGULATION" OF PROTEINS.

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On heating solutions of many proteins, as temperature rises, an irreversible change of state occurs—so-called "heat-coagulation." Similarly, in the case of most proteins endowed with active physiological properties (*e.g.* ferments, toxins, lysins, opsonins, complements, etc.), at or about a particular temperature these properties are destroyed.

Up to the present it has been the almost universal practice to regard the temperature at which change of state or loss of activity occurs, as if it were a physical constant characteristic of the particular protein, this temperature being subject to small variations according to the conditions of experiment.

This may however be an entirely misleading way of regarding the matter and the well-known fact that the destruction of active properties, or precipitation as the case may be, does not occur instantaneously suggests a time process in which heat merely plays the subsidiary part of accelerator. This view of the coagulation of proteins was indeed advanced by Duclaux (1893) and has since been established by Famulener and Madsen (1908) for the destruction of the active properties in solutions of three antigens, vibriolysin, tetanolysin and goats'-serum-hæmolysin, and by Madsen and Streng (1909) for agglutinins.

We have occupied ourselves in the first instance with an investigation of the laws governing the precipitation of solutions of pure proteins because such a process lends itself more easily to accurate quantitative methods.

The fact that various proteins are more or less easily coagulated by heating their solutions has proved useful for their identification and differentiation, see Hewson (1772), Kühne (1864), Fredericq (1877), Weyl (1877), Halliburton (1884).

It was apparent to Weyl that the temperature of heat coagulation, even if it were of the nature of a physical constant, was altered by a variety of conditions and Hammarsten (1879) found that serum globulin, the coagulation temperature of which was determined by Hoppe-Seyler to be 72° to 75° C., coagulated at 68° to 80° C. according to the rate of heating up and the concentration of protein and salt. Osborne and Campbell (1900) found a similar difference in the coagulation temperature of egg albumen by varying the concentration of protein and NaCl. A fourth factor which exerts a profound influence is the addition of even minute amounts of acid or alkali. Halliburton (1884) found in the case of serum albumen that neutralizing (to litmus) an alkaline solution lowered the coagulation temperature from 80° to 78° C. and, by the successive addition of small quantities of acid, it fell as low as 53° C.

The value of the determination of the temperature of heat coagulation as a means of differentiating proteins has been severely criticised by Haycraft and Duggan (1890), who point out that the act of coagulation takes a certain time, and that the temperature of coagulation, as usually studied, rises as the concentration of protein diminishes; it was also criticised by Duclaux (1893). On the other hand, the usefulness of the method when performed under standard conditions was defended by Hewlett (1892), and, provided the four conditions mentioned above be kept uniform, the observation of the temperature at which precipitation commences undoubtedly affords useful information. It will, however, be apparent from the experiments below, that, as insisted upon by Duclaux (1893), heating in solution is not a method which lends itself to the fractional separation of proteins unless the difference in the readiness with which they are thrown out of solution be very considerable. Were the process a reversible one it would be different and the method would compare in usefulness with fractional distillation.

Nature of heat coagulation.

The coagulation of proteins by heating their solutions is not a pure temperature effect. Water as such or in the form of steam is essential. Proteins in the dry or nearly dry condition can be heated to much higher temperatures without change. Cohnheim (1900, p. 141) attributes to Michel and Wichmann the observation that albumen crystals can be heated to 150° C. in the dry condition without change. Again, in the case of proteins soluble in 70 % alcohol, such alcoholic solutions may be boiled without precipitating the protein but, if diluted with water, some coagulation occurs when the solution is heated to the same temperature (Osborne, 1909, p. 21).

The contrast between the effect of heat upon proteins in the dry and moist state is a well-recognised fact, but, with the exception quoted above, we have been unable to discover in the literature any precise experimental data upon the point.

We therefore heated crystalline egg-albumen and methæmoglobin in a hot air-bath jacketted with oil. The proteins were freed as much as possible from moisture by squeezing in a press between filter papers, but still contained 20 % of water. Weighed portions of a few decigrams were placed in muslin bags and hung in the air bath through which a current of air passed. If only small masses are employed, it is not necessary to previously dry the protein, because as long as the residual water is being dissipated the material does not acquire the temperature of the bath. Samples were removed at intervals and estimation made of the protein still soluble in water. We found that crystallised egg-albumen was completely soluble after five hours' heating to 120° C. At 120° C. it was slowly changed and after four hours at this temperature 22 % was rendered insoluble in water. A similar sample in a sealed tube (*i.e.* in the presence of steam at the temperature of the bath) was rendered completely insoluble in a few moments. Methæmoglobin was unchanged by subjection for four hours to a temperature of 110° C. Observations were not made at higher temperatures with this protein.

So-called "heat coagulation" appears to be a reaction taking place between protein and water leading under suitable conditions to the precipitation of the former. If such is the case, it is difficult to imagine that it should occur only at or above any particular temperature, and an interpretation more in accordance with modern chemistry is that heat coagulation is a reaction with a high temperature coefficient, the reaction velocity of which varies considerably with different proteins and according to the acidity and saline content of the solution.

In order to test this interpretation and at the same time to determine the progress of the reaction we kept solutions of two pure proteins, oxyhæmoglobin and crystallised egg-albumen, at constant but different temperatures, removed samples at intervals and determined the amount of protein remaining unchanged. We are alive to the fact that by so doing we are measuring the rate of change in a reaction which involves two distinct processes: (1) the reaction between water and protein, and (2) the separation of the product from a colloidal solution.

Our experiments were performed in the presence of ammonium sulphate which facilitates coherence of the precipitates and, as the filtrates from the partially coagulated solutions displayed no sign whatever of being altered in character and showed no further change on standing at a lower temperature, we believe that, under these conditions, the time occupied in the physical change involved in No. 2 is insignificant and that we are indeed measuring the slower interaction between water and protein.

Materials used. The *hæmoglobin* was prepared from horse's blood and crystallised from solutions of Am_2SO_4, as described by Schulz (1908). The crystals were separated from mother liquor by pressing between filter paper and were dissolved in distilled water. A 3 °/₀ solution of hæmoglobin in a weak solution of Am_2SO_4 was used for the experiments.

Crystalline egg-albumen was prepared from the whites of fresh eggs according to the method of Hopkins and Pinkus (1898). The crystals were pressed as dry as possible between filter paper. A strong solution in distilled water, containing 20·8 °/₀ albumen and 8·4 °/₀ Am_2SO_4 formed the stock solution; it was diluted with the necessary amount of water as required. The stock solution was kept in the cold room as it was not permissible to add a preservative. Even the presence of toluol we found to greatly increase the rate of the reaction.

Method. The protein solutions were placed in a test-tube of 200 c.c. capacity fitted with a stirrer of bent glass tube. The upper end of the stirrer passed through a glass bearing in the cork. Near this upper end a side piece was blown through which the samples were withdrawn. The lower end of this tubular stirrer (Fig. 1) was open, the upper closed and attached to a revolving spindle. The test-tube was immersed in a bath of water with half an inch of mineral oil upon the surface to minimise evaporation. The bath was continuously stirred

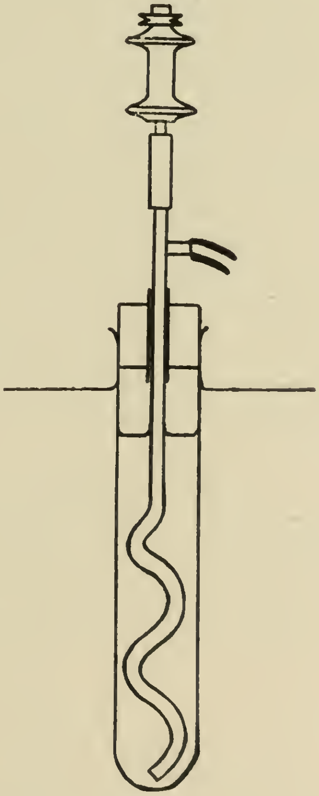

Fig. 1.

and maintained at a constant temperature by means of a gas flame controlled by a toluol regulator. The variations in the temperature of the bath seldom exceeded 0·1° C. during the experiment.

According to size and contents of the tube, 8 to 10 minutes were necessary for the solution to reach the temperature of the bath. A sufficient period having been allowed for warming up, samples of the tube's contents were withdrawn after successive intervals of time, cooled and filtered and the residual proteid determined.

Determination of the proteid content in the samples.

In the case of hæmoglobin, the residual proteid was determined colorimetrically by comparison with the first sample as standard (= 100). This comparison was carried out in a home-made apparatus, which was found to be much more convenient and accurate than any of the more elaborate ones which we tried.

Three test tubes of the same diameter and made of glass of the same tint were carefully selected. The three tubes were observed in a narrow cardboard box, sufficiently long to take them in a row, and deep enough to hold them comfortably. Two square openings exactly opposite one another were cut out in the longer sides of the box, through which the colours could be seen when the box was held up to the light. A cigarette paper was pasted over the opening on the illuminated side.

Two tubes contained the standard solution, diluted as necessary, while a third contained a known volume of the solution to be determined. This was diluted with successive small quantities of distilled water from a burette until its tint could not be distinguished from that of the two standard tubes between which it was placed. A further quantity of water was added until a difference in tint was again noticed. The mean between the readings when a difference could be detected was taken for end-point.

In the case of egg-albumen the concentration of residual unchanged proteid in the filtered samples was determined by boiling a given volume of the filtrate, made just acid with acetic acid, collecting the coagulum on a weighed filter paper, drying at 110° C., and weighing.

The reaction velocity of coagulation.

Hæmoglobin. The hæmoglobin solutions were experimented with as prepared and contained ammonium sulphate. Table I gives the results of five experiments made with a solution of hæmoglobin containing about 3 °/₀ proteid (determined by weighing the coagulum obtained on boiling) at temperatures between 60° C. and 70° C. The residual concentrations of hæmoglobin are expressed in proportion to that of the first sample which is represented by 100. The first sample was taken after sufficient time had elapsed for the tube and contents to take the temperature of the bath, and time is reckoned from the taking of the first sample.

TABLE I. *Coagulation of hæmoglobin, 3 °/₀ solution.*

Exp.	Temp. of bath, ° C.	Time*, minutes $=t$	Concentration of hæmoglobin (first sample=100) $=C$	$\text{Log}_{10} C$	$K=\frac{\log C_0-\log C_n}{t_n-t_0}$
1	60	$0=t_0$	$100=C_0$	2·000	—
		30	54	1·732	·0090
		90	13·5	1·000	·0097
					mean ·0093
2	62·6	$0=t_0$	100	2·000	—
		20	42	1·623	·019
		45	12	1·079	·020
		70	48	·681	·019
					mean ·019
3	65·6	$0=t_0$	100	2·000	—
		10	35·5	1·550	·045
		20	11·0	1·041	·048
		30	5·0	·699	·043
					mean ·044
4	67·6	$0=t_0$	100	2·000	—
		3	61·4	1·788	·071
		6	34·8	1·542	·076
		9	24·9	1·396	·067
					mean ·074
5	70·4	$0=t_0$	100	2·000	—
		2	52·5	1·720	·16
		4	25·3	1·404	·15
		6	14·1	1·150	·15
		7·5	7·6	·886	·15
					mean ·15

* Time was reckoned from the taking of the first sample, *i.e.* from a time when the temperature of tube and contents was known to have reached that of the bath.

In the right-hand column of the table are given values for the velocity constant, calculated on the assumption that the process proceeds logarithmically. These are in good agreement, indicating that the reaction velocity at any moment is proportional to the concentration of unchanged hæmoglobin. This is graphically shown in Fig. 2, where logarithms of hæmoglobin-concentration are plotted against time, and five straight lines are obtained corresponding to the five experiments in Table I. As the temperature is raised, these lines become rapidly steeper, and the velocity constants in Table I become greater in value.

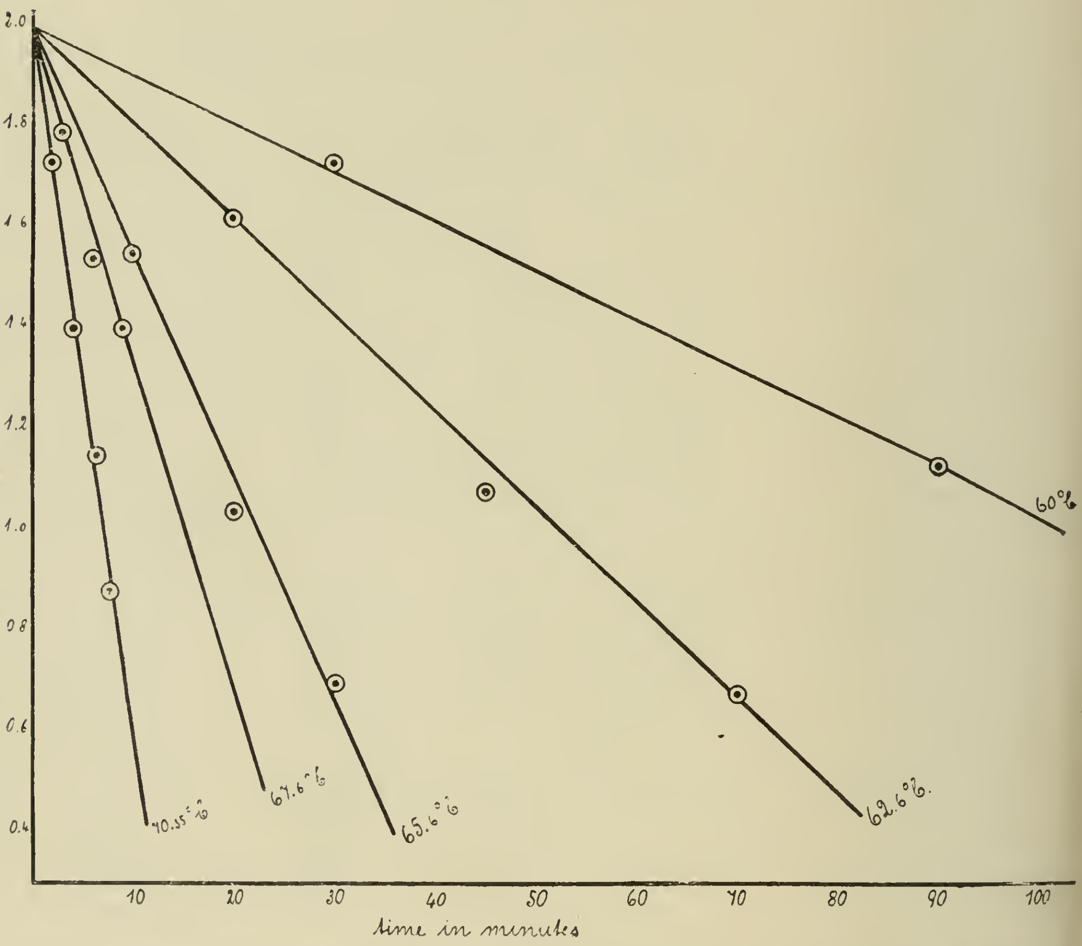

Fig. 2. Coagulation of hæmoglobin in 3 °/₀ solution at temperatures from 60° C. to 70° C. (original concentration = 100). Ordinates = logarithms of concentration of hæmoglobin.

From the results of these experiments it is evident that the change occurring on heating a solution of hæmoglobin is a reaction of the first order.

Our interpretation of the facts is as follows: Water reacts with hæmoglobin, and this reaction results in the splitting off of hæmatin and the formation of an insoluble protein. As the water is in infinite excess, at any one temperature the rate of reaction is determined only by the residual concentration of the hæmoglobin. At ordinary temperatures this reaction between hæmoglobin and water is inappreciable, but is greatly enhanced by raising the temperature, in other words the reaction has a high temperature coefficient. This has been determined for coagulation of hæmoglobin and egg-albumen and will be discussed later.

What the nature of the cleavage may be we are ignorant but the phenomenon presents some analogy with the hydrolysis of cane sugar.

Crystallised egg-albumen. A 1 °/₀ solution of egg-albumen was used and the experiments were similar to those with hæmoglobin detailed above, except that the concentration of albumen in the samples removed at intervals was determined by boiling and weighing the precipitated protein. The temperatures employed varied between 69° and 77° C.

The crystals of egg-albumen were twice recrystallised from solutions of ammonium sulphate. The solutions contained free acid and required the addition of considerable quantities of alkali before the concentration of H^+ ions was reduced to that of water. The observations were made by determining the electromotive force of hydrogen concentration-cells and will be referred to again later. The fact is mentioned here to indicate that egg-albumen crystals are salts of the protein with the acid present at the time of their precipitation as was found by Osborne (1902) to be the case with Edestin crystals. Such salt formation by proteins has been abundantly shown among others by Skojvist (1895), Spiro and Pemsel (1898), Osborne (1899 I and II and 1902), Erb (1901), Pauli (1907), Moore and Bigland (1910). In water these salts hydrolyse to some extent and a solution containing salts of albumen, albumen and acid is produced. Under these circumstances, it might be anticipated that the progress of the reaction with hot water would be more complicated than was the case with hæmoglobin.

In Table II are set forth the results of four experiments showing the progress of coagulation in a 1 °/₀ solution of egg-albumen crystals at temperatures of 69° to 76·3° C. The solution was rendered alkaline to lacmoid solution by the addition of 5 c.c. $\frac{N}{10}$ AmOH to 100 c.c.[1] and subsequent examination of the concentration of H^+ ions in this solution with a gas-chain battery by the hydrogen concentration-cell method indicated that the solution was only slightly more alkaline than distilled water. Nevertheless, a distinct formation of alkali albumen was detected after the solution had been heated for some hours. The residual concentration of protein in the solutions is given in milligrams per c.c.

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[1] The original albumen solution contained 1·039 °/₀ protein, so that the amount of ammonia added is equivalent to 4·81 c.c. $\frac{N}{10}$ AmOH per gram protein; after this dilution the solution contained 0·99 °/₀ albumen.

TABLE II. *Coagulation of* 0·99 $^0/_0$ *crystalline egg-albumen solution.*

Exp.	Temp. of bath, °C.	Time, minutes	Amt. of filtrate analysed, c.c.	Weight of coagulum, grams	Residual albumen, mgrs. per c.c.
1	69	10	15	0·1462	9·747
		85	15	0·1104	7·360
		180	14·5	0·0838	5·779
		360	14·3	0·0598	4·184
		741	21	0·0585	2·786
		1443	30	0·0604	2·013
2	71·1	11	13·5	0·1326	9·823
		37	17·2	0·1162	6·756
		62	11·7	0·0607	5·187
		90	18	0·0850	4·722
		146	19·5	0·0634	3·251
		280	23	0·0487	2·117
3	73	10	14·5	0·1379	9·512
		20	14·5	0·0818	5·642
		30	12·5	0·0545	4·360
		50	16	0·0470	2·938
		129	17·5	0·0270	1·543
		275	34	0·0393	1·156
4	76·3	10·5	12	0·0442	3·683
		13	12	0·0312	2·600
		17	20	0·0407	2·035
		22	18	0·0243	1·350
		30	40	0·0480	1·200

In Fig. 3, the concentration after definite intervals is plotted against time. The points appear to lie upon smooth curves, indicating that the process proceeds in an orderly manner. An examination of the figures showed that the relation of the concentration to time did not follow a simple logarithmic law, but decreased with time more quickly than would be accounted for if the rate were merely proportional to the concentration of protein.

We have made a large number of experiments with egg-albumen solution, the details of which we have not included in this paper. The results of these experiments showed general agreement with those described above and afforded curves similar to those drawn in Fig. 3. For some time we were not aware how extremely sensitive this process is to a variety of influences, and that it is essential to work with the same solution if the results are to be compared. No preservative is permissible and the solutions must be frozen during the time between the observations. In the early portion of the work sufficient precautions were not taken, and these experiments cannot therefore be used for comparative purposes.

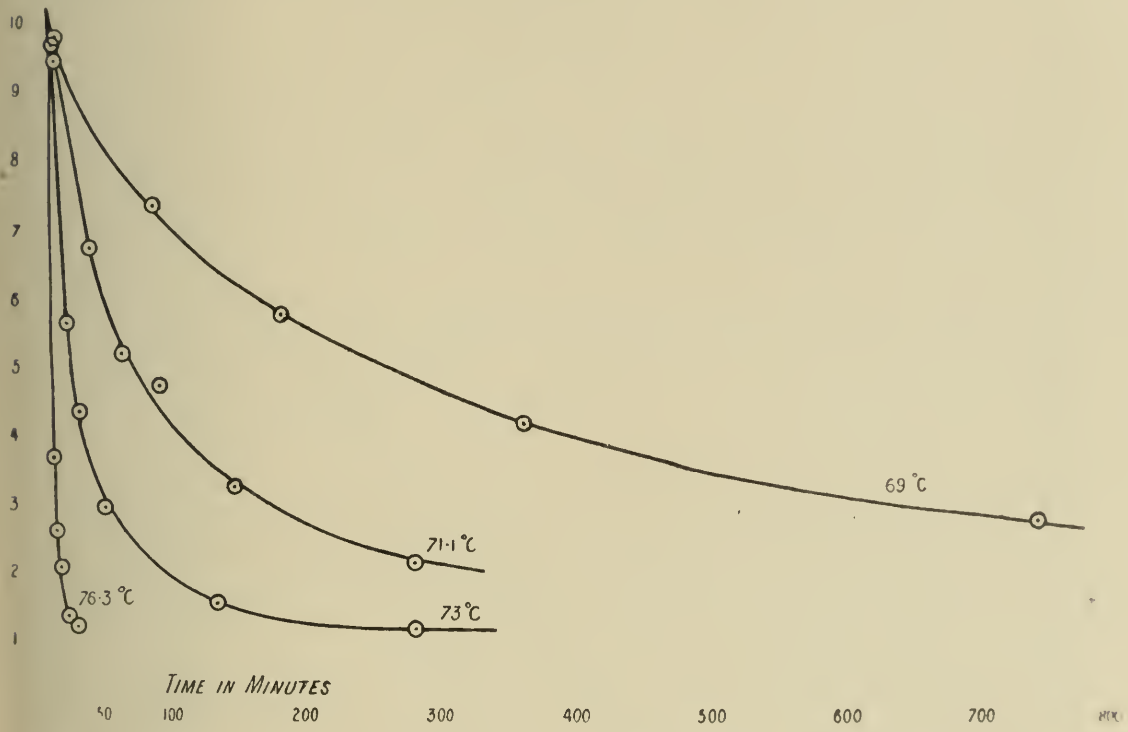

Fig. 3. Influence of temperature upon coagulation rate of egg-albumen. Coagulation of 1 °/₀ solution at temperatures from 69° C. to 76·3° C. Ordinates = concentration of albumen in milligrams per c.c.

TABLE III. *Temperature coefficient of heat coagulation of hæmoglobin.*

Material	Temperature of bath $=t$	Velocity constant $=K$	Log_{10} $(K\times 10^3)$ $=\log k$	Mean logarithmic difference in velocity constant per 1° C.	Temperature coefficient, mean relative change in velocity constant for 1° C.	$\mu = \frac{2T_0T_n}{T_0-T_n}(\log_e K_0 - \log_e K_n)$
Hæmoglobin	70·4 $=t_0$	0·15 $=K_0$	2·176 $=\log k_0$	—	—	—
	67·6	0·074	1·869	0·109	—	58,700
	65·6	0·044	1·643	0·111	—	59,410
	62·6	0·019	1·278	0·115	—	61,030
	60	0·0093	0·968	0·116	—	61,070
				mean 0·113	1·30	mean 60,050

Temperature coefficient of the reaction.

Hæmoglobin. The effect of temperature upon the reaction velocity of coagulation was studied by comparing the velocity constants at the various temperatures. The available data obtained from Table I are set forth in Table III, and it will be seen that between the temperatures of 60° C. and 70° C., rise of temperature has a consistent effect upon the rate of coagulation. This is in approximate obedience to a logarithmic

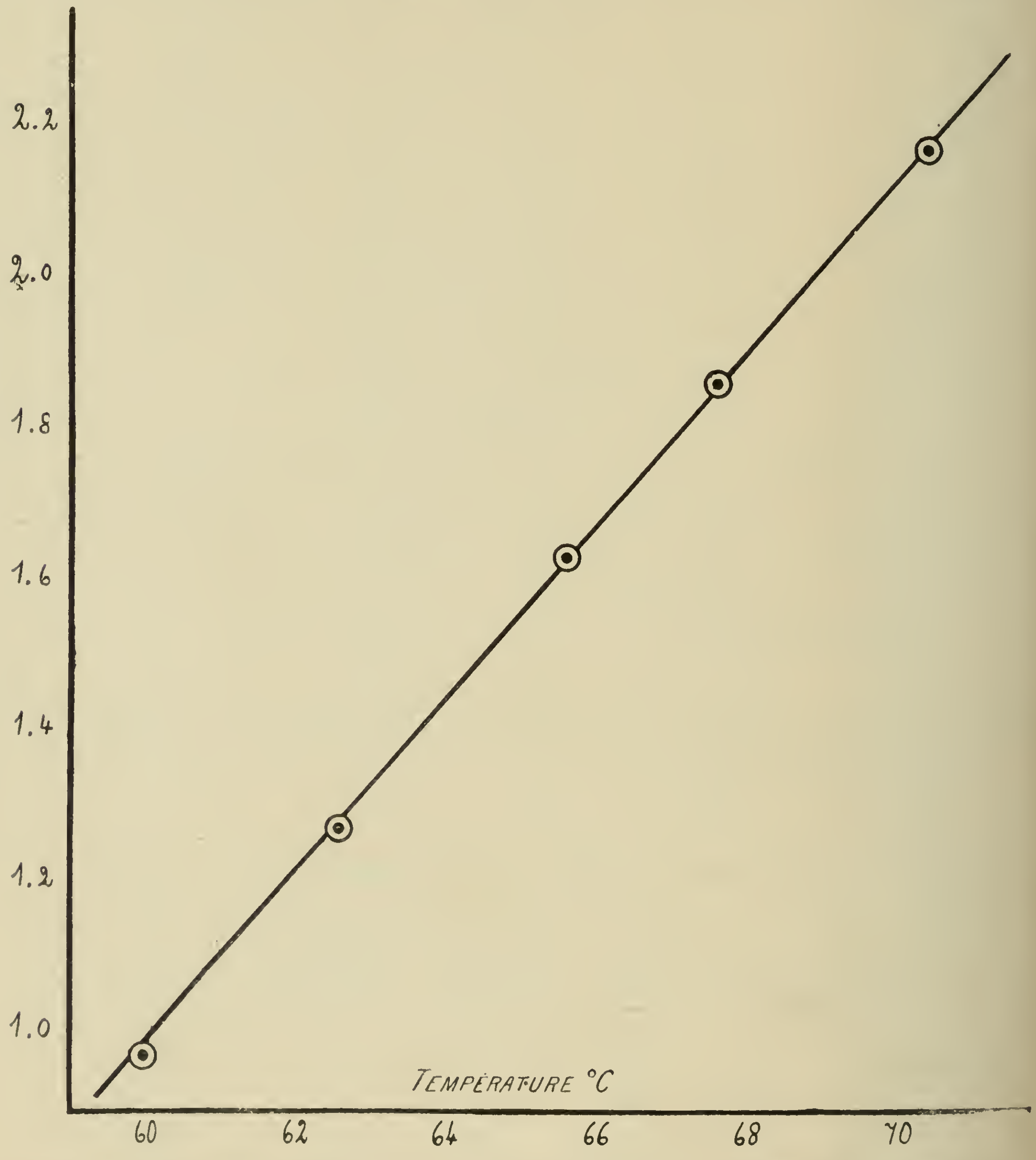

Fig. 4. Influence of temperature upon the reaction velocity of hæmoglobin coagulation (3 % solution), logarithms of mean values of the velocity constant (ordinates), K, plotted against temperature (abscissæ).

law, the mean logarithmic difference in velocity constant for a rise of temperature of 1° C., calculated from the various different experiments, remaining nearly constant in value throughout the range observed. This is expressed graphically in Fig. 4, where logarithms of velocity constants, plotted against temperature, fall upon a straight line.

The formula of Arrhenius, in which an additional factor having reference to the absolute temperatures is introduced, has, in many cases, been found to express the effect of temperature upon reactions, and would appear to be applicable here equally with the simpler law discussed in the preceding paragraph. The values of $\mu \left(= \frac{2T_o T_n}{T_o - T_n} \log_e \frac{K_o}{K_n}\right)$ are given in the last column of Table III, and a fair approximation to constancy is displayed. Over the small range of temperature of the experiments, it is difficult to say which expression is the better applicable.

From Table III the coagulation rate of hæmoglobin is seen to be increased 1·3 times for a rise in temperature of 1° C., *i.e.* 13·8 times for a rise in temperature of 10° C.

Egg-albumen. It is not possible, as was done in the case of hæmoglobin, to ascertain the temperature coefficient by a direct comparison of velocity constants at different temperatures, because the equation to the curve is unknown. It is legitimate, however, to compare the time taken for a similar reduction in concentration of unaltered albumen at various temperatures. In Fig. 3 concentrations of residual albumen are plotted against time, at four different temperatures; from the smoothed curves the times taken at each temperature for a concentration of albumen (A) to be reduced to concentration (B) can be determined. The reciprocals of these times are proportional to the average velocities of similar portions of the total coagulation process at the various temperatures and may be compared. In this way the figures in Table IV are obtained, giving the times taken for reduction of concentration of uncoagulated albumen from 9 mgr. per c.c. to 3 mgr. per c.c. in each case.

Rise of temperature within the limits employed has a consistent influence upon the average velocity of coagulation of egg-albumen and this influence is even greater than was the case with hæmoglobin. The average velocity of coagulation of egg-albumen is increased 1·91 times for a rise in temperature of 1° C., *i.e.* at the rate of about 635 times for 10° C.

TABLE IV. *Influence of temperature upon the coagulation of crystalline egg-albumen.*

Temp., ° C. = t	Time taken for reduction, 9 mgrs. per c.c. to 3 mgrs. per c.c., minutes	Average velocity during this period, mgr. per c.c. coagulated per minute = V	Log_{10} ($V \times 10^3$)	Mean logarithmic difference in average velocity per 1° C.	Temperature coefficient, mean relative change in average velocity per 1° C.	$\mu = \frac{2T_0 T_n}{T_n - T_0}(\log_e V_n - \log_e V_0)$
69·0 = t_0	617·0	0·00973 = V_0	0·988	—	—	—
71·1	155·0	0·03871	1·587	0·285	1·93	136,500
73·0	41·5	0·1446	2·160	0·294	1·97	141,600
76·3	7·2*	0·833	2·921	0·265	1·84	128,800
				Mean	1·91	

* Figure by extrapolation.

In the last column of Table IV are given the value of μ, if the formula of Arrhenius be supposed to hold in this instance. The value obtained from the various experiments shows a rough constancy and is nearly three times that obtaining in the case of hæmoglobin.

The temperature coefficient which we find for the coagulation of these two proteins by hot water, 1·30 and 1·91 times respectively for 1° C. rise in temperature, is an exceedingly high one compared with the effect of temperature upon most chemical reactions. In the majority of instances the reaction velocity is increased about 1·1 times for 1° C., that is 2 to 3 times for a rise of temperature of 10° C., and even the biological processes of germination of seeds, respiration of plants and growth of bacteria fall within this range. On the other hand, many reactions in which complex protein bodies are concerned have been shown to possess high temperature coefficients which are comparable with those obtained for heat coagulation. The destruction of emulsin by heat has, according to Tamman (1895), a temperature coefficient of 7·14 for 10° C. rise of temperature between the temperatures of 60° C. and 70° C. Bayliss (1908, p. 52) found the action of trypsin to be hastened 5·3 times for 10° C. rise of temperature. Meyer (1906) found the disinfection of spores of B. subtilis and B. robur by heat to be hastened from 4 to 5 times for 10° C. rise of temperature, in accordance with a logarithmic law. Ballner (1902) found disinfection of anthrax spores by steam to take place from 9 to 11 times more quickly by raising the temperature 10° C.; a simple logarithmic law and the law of Arrhenius are equally applicable to his results (H. Chick, 1908, p. 153). Disinfection of vegetative forms of bacteria (B. paratyphosus) with phenol and other coal tar derivatives has a temperature coefficient of 8 to 10 for 10° C. rise in temperature[1].

The high temperature coefficient for coagulation of egg-albumen has a counterpart in that for the velocity of destruction by hot water of the hæmolysins in vibriolysin, tetanolysin and goat-serum. Famulener and Madsen (1908) found the influence of temperatue to be in accordance with the law of Arrhenius, and the velocity of this reaction to be doubled if the temperature were raised 1° C. Madsen and Streng (1909) showed the action of hot water upon some agglutinins to be similarly influenced by temperature.

All the high temperature coefficients mentioned above have been observed with reactions taking place in colloidal solutions which suggests that under these conditions the effect of temperature is produced in some other way than by merely enhancing the intrinsic energy of the molecules. In what other way temperature may operate, we have however no proof. Mr W. B. Hardy has suggested to us that alteration in the mean size of the protein aggregates, whereby the total surface was increased, might explain a rise in reaction velocity. The heat coefficient of egg-albumen may be in part so explained, but, since from the osmotic pressure determinations of Hüfner and Gansser (1907) and Roaf (1909) hæmoglobin would seem to exist in solution in single molecules, some other interpretation must be looked for in this case.

It must not be overlooked that in all these observations the range over which the influence of temperature has been studied is a very small one—in our own experiments 10° C.—and there is no experimental proof that the effect of temperature remains the same over a wider range. We have, however, observed the formation of an insoluble precipitate in faintly acid solutions of egg white after one week at 37° C., and it is a matter of common observation that protein solutions slowly deposit an insoluble precipitate of protein on long standing.

From the results set forth in this paper it is clearly inaccurate to consider that a protein coagulates at any particular temperature. It is true that proteins heated with water under exactly similar circumstances usually commence to precipitate at or about some definite temperatures, but it is misleading to look upon the "coagulation temperature" as if it were of the nature of a physical constant of the particular protein. It is due to the high temperature coefficient of this reaction that observations of the so-called coagulation temperature have been of service. A real distinction is, however, afforded by the *rate* at which a protein is coagulated at any given temperature and under similar conditions as to reaction and salt content.

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[1] On the other hand disinfection by mercuric chloride or silver nitrate has a much lower coefficient, viz. 2—4, (H. Chick (1908), B. paratyphosus; Madsen and Nyman (1907) anthraxspores).

Effects of acid and alkali upon rate of coagulation of egg-albumen.

It has long been known that the addition of acid or alkali influences the readiness with which protein is thrown out of solution by hot water. Halliburton (1884) found that with solutions alkaline to litmus, the temperature at which precipitation occurred, if at all, was not much influenced by the degree of alkalinity. On the other hand, acid lowered the temperature of coagulation; if acid were added in equal quantity to a solution of albumen the first additions produced a small fall in coagulation temperature, and this effect became progressively more marked with each successive addition. Halliburton's results as to the general effect of acid in increasing the readiness with which proteins were precipitated from their solutions were confirmed by Haycraft and Duggan (1890).

A certain amount of confusion upon this subject is due to the fact that in the past the criterion of neutrality has been arrived at with the use of indicators, substances which in many cases act as stronger acids or bases than the proteins themselves. Our observations with solutions of egg-albumen have led us to the conclusion that directly the concentration of OH^- ions in a solution rises above a minimum (about twice the concentration in distilled water) some alkali-albumen is formed on heating. The question therefore resolves itself into the effect of acid.

Having ascertained that it is improper to speak of a protein as having any particular coagulation temperature, we have studied the effect of addition of acid upon the velocity of coagulation at constant temperature. For this purpose hæmoglobin could not be used owing to its extreme sensitiveness to acid and the experiments were therefore confined to crystalline egg-albumen.

In Table V are given the results of six experiments made with the same material and at the same temperature, 69° C., and differing only in the reaction of the albumen solution employed, which varied from the material as prepared, with markedly acid reaction, to the limit

TABLE V. *Coagulation of crystalline egg-albumen,* 0·99 $^0/_0$ *solution, with original acidity, and after addition of various amounts of ammonia, at* 69° C.

Exp.	No. of c.c. $\frac{N}{10}$ AmOH added per gram protein	Time, minutes	Amount of filtrate analysed, c.c.	Weight of coagulum, grams	Residual albumen, mgrs. per c.c.
1	0	0	control	—	9·901
		8	22	0·1262	5·737
		10	23	0·1022	4·444
		11	24·5	0·0989	4·037
		12·5	22·5	0·0800	3·555
		15	34·5	0·0999	2·896
2	1·60	10	16	0·1260	7·876
		15	16·5	0·1105	6·697
		22	22·5	0·1257	5·587
		30	23	0·1111	4·831
		50	32	0·1172	3·663
		80	18	0·0506	2·811
3	2·40	10	17	0·1461	8·594
		20	16	0·1168	7·301
		40	20	0·1180	5·900
		81	18	0·0795	4·416
		150	22·5	0·0806	3·582
		337	28·5	0·0716	2·512
4	3·20	10	17	0·1458	8·576
		20	15·5	0·1246	8·040
		40	8·5	0·0636	7·482
		86	30	0·1809	6·030
		165	21	0·1025	4·881
5	3·53	10	12·7	0·1201	9·458
		30	15·5	0·1279	8·251
		60	16	0·1144	7·151
		120	18·5	0·1058	5·719
		241	18·7	0·0802	4·288
		420	14·8	0·0428	2·892
		600	36	0·0709	1·969
6	4·01	10	13	0·1230	9·461
		40	12·5	0·1000	8·001
		90	16	0·1073	6·707
		215	17·5	0·0836	4·777
		300	20	0·0810	4·050
		645	24	0·0589	2·454
7	4·81	See Table III, Experiment at 69° C.			

of alkalinity possible without formation of alkali albumen[1] (Exp. 7). This is after all a very small range, but was the utmost within the compass of our experimental method for any one temperature. As it was, the reaction rate in the most acid condition (Exp. 1) was so rapid as to make the observations only just possible, whereas the experiment with neutral solution occupied 12 hours.

The results are shown graphically in Fig. 5 where the concentration of residual albumen in solution for seven different degrees of acidity are plotted against time. The observations fall upon smooth curves. As in

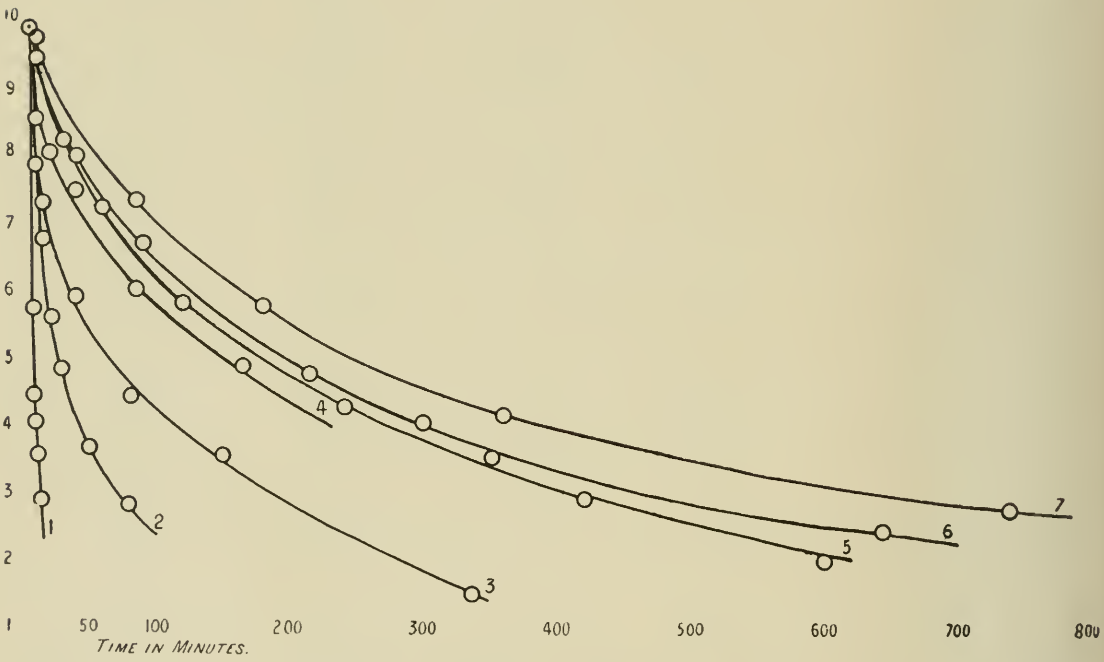

Fig. 5. Influence of acid upon coagulation rate at 69° C. of egg-albumen in 1 $^0/_0$ solutions to which varying amounts of alkali had been added. Ordinates = concentration of albumen in milligrams per c.c. Abscissæ = Time minutes.

1. Original solution, reaction acid, *i.e.* H^+ ion concentration greater than distilled water.
2. 1·6 c.c. $\frac{N}{10}$ AmOH added per gram protein, reaction acid, ,, ,,
3. 2·4 ,, ,, ,, ,, ,, ,,
4. 3·2 ,, ,, ,, ,, ,, ,,
5. 3·53 ,, ,, ,, ,, ,, ,,
6. 4·01 ,, ,, ,, reaction alkaline, *i.e.* H^+ ion concentration less than distilled water.
7. 4·81 ,, ,, ,, ,, ,, ,,

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[1] It must be remembered that the original solution of twice re-crystallised egg-albumen crystals is decidedly acid and that we have studied the effect of varying the acidity by adding different amounts of alkali. This method was employed owing to the fact that at that time we had no means of ascertaining the true neutral point of the solution. It has no disadvantage other than rendering the description involved.

the previous series the reaction rate falls off more rapidly than can be accounted for by the decrease in concentration, but the nature of the curve has not been ascertained.

Therefore, for a quantitative comparison of the rates of coagulation with different degrees of acidity the same expedient had to be adopted as before and the average velocities derived from the times taken for the concentration to be reduced from 6 mgrs. per c.c. to 3 mgrs. per c.c. in each case. The figures were obtained from the smoothed curves of Fig. 5, and are set forth in Table VI.

Examination of Table VI or a glance at Fig. 6, in which the mean velocity of coagulation is plotted against no. of c.c. $\frac{N}{10}$ alkali added per gram protein, reveals the fact that as the acid is removed the rate of coagulation falls, at first very rapidly, afterwards more slowly, the effect being in no simple proportion to the alkali added.

TABLE VI. *Effect of acid upon rate of coagulation of a* 0·99 % *solution of crystalline egg-albumen. Total volume of solution constant* = 157·5 *c.c.*

Composition solution no. of c.c. $\frac{N}{10}$ AmOH added per gram protein	Reaction of solution	Time taken for reduction in albumen concentration of 6 mgrs. per c.c. to 3 mgrs. per c.c., mins. (from Fig. 5)	Average velocity during this period mgrs. per c.c. coagulated per minute
0	Original acidity	7·1	·4225
1·60	Acid	57·5	·0522
2·40	,,	150	·0200
3·20	,,	240	·0125
3·53	,,	300	·0100
4·01	alkaline	370	·00811
4·81	,,	480	·00625

These results confirm in the main those of Halliburton (1884) with serum proteins already referred to (p. 405). In our experiments the effect of acid is more striking, but that is to be explained as follows. The influence of acid is here measured by comparing coagulation rates at constant temperature, whereas Halliburton compared the "coagulation temperatures," that is to say temperatures at which coagulation takes place at approximately the same rate. For a process with a high temperature coefficient the former method is the more accurate. An amount of acid which would double the coagulation rate at a given temperature would lower the apparent "coagulation temperature" only about 1° C.

Little further could be made of the facts until we had found out the true neutral point of the solution, *i.e.* the point where the concentration of hydrogen ions equalled that in distilled water, and had also determined what influence the withdrawal of acid was producing upon the real acidity of the solution (concentration of hydrogen ions).

This we have been enabled to carry out owing to the kindness of Dr N. T. M. Wilsmore of University College, who not only placed the resources of his laboratory at our disposal but with infinite pains instructed us in the use of the gas-chain battery and generally looked after us while we made the observations. Subsequently we installed the apparatus in our own laboratory and repeated the determinations.

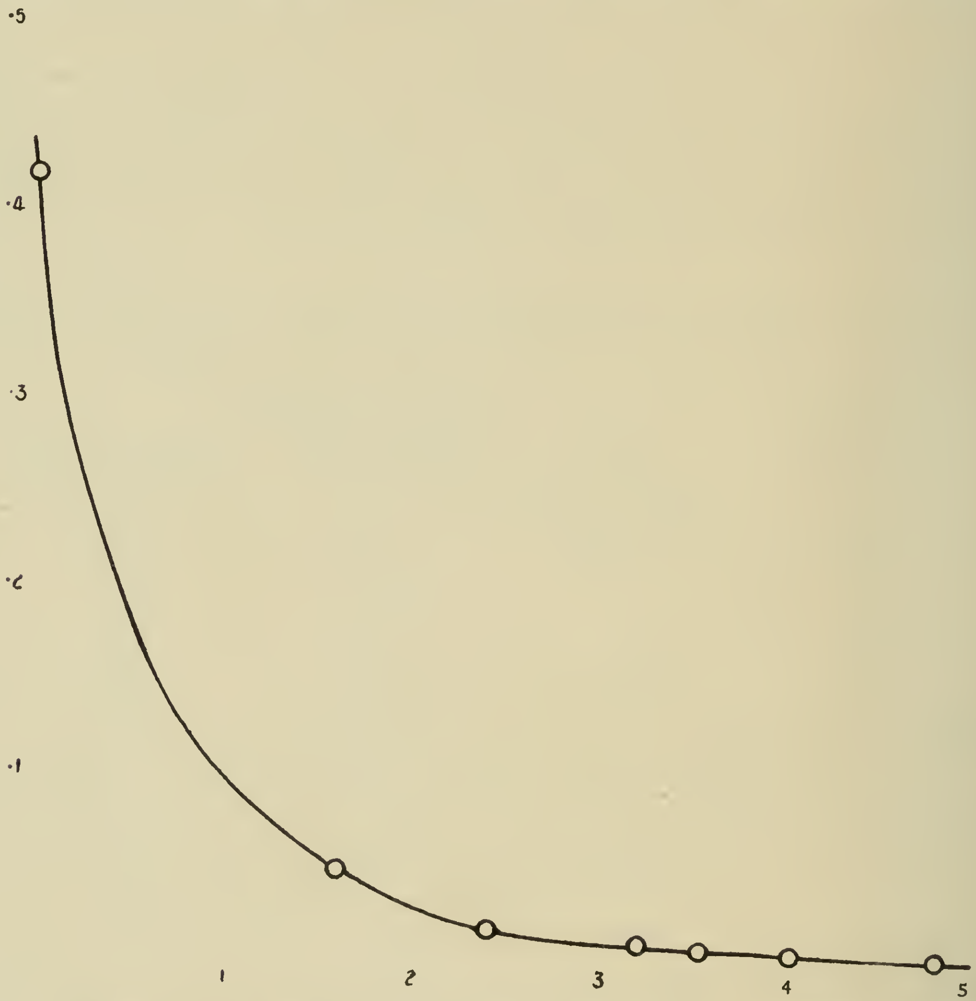

Fig. 6. Influence of acid upon coagulation rate of egg-albumen. Ordinates = milligrams per c.c. coagulated per minute. Abscissæ = number of c.c. $\frac{N}{10}$ AmOH added to the original acid solution per gram protein.

The particular deposition of the apparatus we used was that described by Wilsmore (1900). Two hydrogen electrodes were employed; one dipped into $\frac{N}{10}$ HCl, and the other into the solution whose

E.M.F. was to be measured. The two cells were connected by a vessel containing a saturated solution of ammonium nitrate, in order to minimise the contact potential between the two solutions. The temperature was maintained at 18° C. The solutions under investigation contained 1 °/₀ egg-albumen, 0·4 °/₀ ammonium sulphate and varying quantities of $\frac{N}{10}$ AmOH. After adding the alkali the solutions stood overnight in order that equilibrium might be attained.

We first ascertained that the E.M.F. given when one cell contained $\frac{N}{10}$ HCl and the other $\frac{N}{1000}$ HCl was nearly the theoretical value, viz. ·1154 volt. In the series of observations detailed in the table the actual value obtained under these conditions was 0·1150 volt. The small error due to the presence of 0·4 °/₀ Am_2SO_4 in one cell only was subsequently ascertained to be + ·004 volt and in the table the observed values for E.M.F. have been corrected by this amount.

TABLE VII. *Concentration of* H^+ *ions in* 0·99 °/₀ *solution after addition of various quantities of* $\frac{N}{10}$ AmOH. *Total volume of solution constant.*

Solution in contact with 1st H. electrode,		Solution in contact with 2nd H. electrode, c.c. $\frac{N}{10}$ AmOH added per grm. protein	E.M.F. volts (corrected)	Calculated concentration of H^+ ions
$\frac{N}{10}$ HCl		0·00	—	251×10^{-7} N.
		0·20	0·211	$222\times{}^{-7}$ N.
		1·20	0·238	75·0 ,,
		2·20	0·263	27·7 ,,
	Acid	2·84	0·294	7·96 ,,
	↑	3·17	0·316	3·33 ,,
		3·53	0·334	1·62 ,,
		3·60	0·344	1·09 ,,
	Neutral ↓	4·35	0·360	0·58 ,,
	alkaline	4·80	0·369	0·41 ,,

The results of these observations are set out in Table VII, where the number of c.c. $\frac{N}{10}$ AmOH added per gram of proteid, the E.M.F. observed and the concentration of hydrogen ions corresponding thereto appear in parallel columns. The calculation of the concentration of the hydrogen ions is made by means of the general formula of Nernst for the case of similar electrodes and solutions containing univalent ions, viz;

$$\text{E.M.F.} = 0{\cdot}0577 \log_{\cdot 10} \frac{\text{concentration } H_1^+}{\text{concentration } H_2^+}.$$

The results permit of important conclusions regarding the composition of our egg-albumen solution. The 1 °/₀ solution of egg-albumen crystals, as originally made up, contained a concentration of free acid of 251×10^{-7} normal, and required the addition of 4·0 c.c. $\frac{N}{10}$ AmOH to reduce that concentration to that of pure water, so that the total acid combined with 1 gram of protein as crystals was 0·0004 equivalents and of this amount 0·7 °/₀ was free in the solution. Egg-albumen crystals, crystallised from acetic acid ammonium sulphate solutions were shown by Osborne (1899) to consist to some considerable part of protein combined with acid to form a salt or series of salts. What in our solutions was the nature of the acid we have not determined, but, as they were prepared in the first instance from a solution of egg-white (containing excess of ammonium sulphate) and subsequently twice recrystallised from strong ammonium sulphate solutions, it is probable that the acid radicle was SO_4[1]. The amount of hydrolysis is surprisingly small, when we consider that the solution cannot be stronger than about $\frac{1}{300}$th normal and is probably less, but, as we are at present ignorant how far the protein is saturated with acid, it is impossible to draw any conclusions as to the strength of egg-albumen as a base.

Our observations do not afford any simple explanation of the effect of acid upon the coagulation rate of a protein solution. Having ascertained that "heat coagulation" was a reaction between the protein and water in which heat and acid play the subsidiary part of accelerators we thought that, as is the case with the hydrolysis of sugar, the velocity of the reaction might be conditioned by the concentration of hydrogen ions present. At constant temperature the rate is, however, not entirely dependent upon their concentration, as $\frac{\text{velocity}}{\text{H}^+ \text{ concentration}}$ is not a constant. In Fig. 7 the logarithms of the mean coagulation rate at 69° C. (Table VI) and the logarithms of the hydrogen ion concentration are both plotted against the alkali added per gram of protein, but, although indicating a certain parallelism in the mediate portion of the curve, there is clearly another factor involved. At first (*i.e.* near the neutral point) the hydrogen ion concentration rises much more quickly than the coagulation rate, then becomes nearly proportional and finally the rate of coagulation increases more rapidly than the concentration of the hydrogen ions.

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[1] Osborne (1899, II) has shown that crystals of Edestin prepared in an analogous manner in the presence of NaCl contain the chloride.

It may well be that the apparent relation, such as it is, of coagulation rate to concentration of hydrogen ions is a spurious one and that the gradual conversion of the protein to protein salt, or to a series of protein salts of increasing acidity and different velocity coefficients accounts for the influence of acid upon the rate of coagulation.

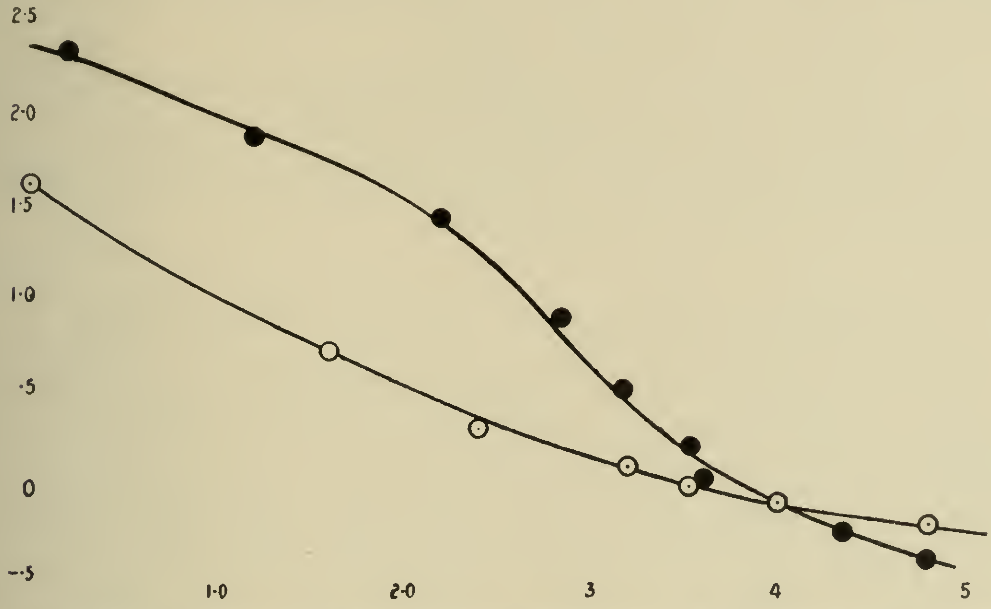

Fig. 7. Relation between average velocity of egg-albumen coagulation and the concentration of free acid (H^+ ions) in the solution.

● = hydrogen ion concentration, ordinates = $\log_{10}$ (concentration of H^+ ions × 10^7).

○ = average velocity of coagulation, ordinates = $\log_{10}$ (average velocity of coagulation × 10^2).

Abscissæ = number of c.c. $\frac{N}{10}$ AmOH added per gram protein to the original acid solution.

TABLE VIII. *Effect of acid upon coagulation rate of a* 1·003 °/₀ *solution*[1] *of egg-albumen, comparison of sulphuric and acetic acids.*

Temp. = 70° C., time = 55 minutes.

Composition of solution	Amount of filtrate after coagulation taken for analysis, c.c.	Weight of coagulum, grams	Uncoagulated protein, mgrs. per c.c. filtrate	°/₀ coagulated
Control, 10 c.c. albumen solution + 1 c.c. H_2O	—	—	91·2	—
10 c.c. albumen solution + 0·33 c.c. $\frac{N}{10}$ H_2SO_4 + 0·6 c.c. H_2O	4·40	0·0224	50·9	44·2
10 c.c. albumen solution + 0·16 $\frac{N}{10}$ H_2SO_4 + 0·16 $\frac{N}{10}$ HA + 0·66 c.c. H_2O	4·81	0·0243	50·5	44·6

[1] This solution has been made neutral to litmus with ammonia.

We thought to throw light upon this question by comparing the effect of equivalent quantities of sulphuric acid and acetic acid upon coagulation rate. From Table VIII it will be seen that the effect of the weaker and stronger acids was almost the same. At first this appeared to settle the matter but on calculating the concentration of free acid (about $N \times 10^{-5}$) it was of such a dilution that even acetic acid may be considered to be completely ionised.

We cannot therefore decide to what extent the influence of acid upon the reaction rate is to be attributed to variation of hydrogen ion concentration, and to alteration in the nature and proportion of salts of protein in the solution respectively.

Diminution of free acid on coagulation of egg-albumen.

It is common experience that on heating a protein solution, previously rendered neutral or faintly acid to litmus paper, it becomes progressively less acid or even alkaline *to litmus paper* as coagulation proceeds. Due allowance for this phenomenon was made by Halliburton (1884, p. 155) when using the fractional coagulation method for separating the proteins of serum. Corin and Bérard (1888) were also aware of the phenomenon and sought to compensate[1] for it in their endeavour to differentiate the proteins in egg-white. Haycraft and Duggan (1890) suggested that the phenomenon might account for the continual "raising of coagulation temperature" during the progress of coagulation.

The observations detailed in a previous part of this paper confirming those of Spiro and Pemsel (1898), Osborne (1899), and Erb (1901), show that, when acid in not too great an amount is added to solutions of crystalline egg-albumen, the greater proportion is immediately combined, but a small amount remains free owing to the hydrolysis of the salt.

The fate on coagulation of this free acid was quantitatively investigated as follows: A series of albumen solutions were prepared, all containing approximately 1 °/₀ crystalline egg albumen, but possessed of different degrees of acidity. The concentration of H^+ ions in these solutions was determined both before and after the solutions had been heated to 90° C. for 5 minutes. The results are given in Table IX., which shows that for small concentration of acid, up to $\frac{1}{10,000}$ normal, the amount withdrawn from the solution is nearly proportional to the initial concentration of free acid. Above this strength the acid removed becomes proportionally less.

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[1] They attempted to adjust the reaction of the filtrate from each successive coagulation to the same point by first adding NaOH until it just showed pink with phenol-phthalein and then adding a constant amount of acid per unit volume. This procedure would be successful were it not for the fact that the protein content varied in each successive filtrate. Corin and Bérard were unaware of the salt formation of proteins, and did not realise that in consequence solutions in which their successive fractions were coagulated were progressively more and more acid. This no doubt explains partly the difference in the conclusions arrived at by these authors and other observers.

The relation has not been further investigated, but is not unlike that found in absorption phenomena. We found that egg-albumen precipitated by heating in nearly neutral solution, washed and added to $\frac{1}{3000}$ normal HCl removes acid from the solution.

TABLE IX. *Showing the amount of acid fixed during coagulation of 0·99 % albumen solutions of differing acidity.*

Solution in contact with 1st H electrode	Solution in contact with 2nd H electrode	E.M.F., volts		Calculated concentration of H+ ions in albumen solution		Calculated equivalents of acid fixed per gm. protein
		As made up	After heating	As made up	After heating	
$\frac{N}{10}$ HCl	No. 1 alb. sol.	·3010	·3451	$6·07 \times 10^{-7}$ N.	$1·04 \times 10^{-7}$ N.*	$0·51 \times 10^{-7}$
	No. 2 ,,	·2422	·3100	63·4 ,,	4·24 ,,	5·98 ,,
	No. 3† ,,	·1634	·2051	1470 ,,	279 ,,	131 ,,
	No. 4† ,,	·1097	·1192	12600 ,,	8590 ,,	459 ,,

* Neutrality is equivalent to $·8 \times 10^{-7}$ N.

† Solutions 3 and 4 contained ·91 % and ·87 % albumen respectively.

SUMMARY AND CONCLUSIONS.

The process of "heat coagulation" has been studied with solutions of crystallised hæmoglobin and crystallised egg-albumen. The complete solubility of both proteids after exposure to dry heat at high temperatures (110° C. – 130° C.) indicates that "heat coagulation" of protein solutions is not a pure temperature effect, but a reaction between water and protein.

In the case of solutions of hæmoglobin the rate of coagulation at any moment is proportional to the concentration of residual hæmoglobin, *i.e.* it is a reaction of the first order.

Coagulation of solutions of egg-albumen crystals is also an orderly time process, but the rate decreases more rapidly as coagulation proceeds than can be accounted for by the decrease in concentration of uncoagulated protein. The explanation of this increased complexity is attributed to want of homogeneity in the composition of egg-albumen crystals, and to the changing conditions due to the absorption of free acid by the coagulum as formed, see below.

The conclusion of Osborne that egg-albumen crystals as prepared by Hopkins' method consist of salts of protein with the acids used in their preparation is confirmed.

In the case of 1 % solutions of egg-albumen, twice recrystallised from ammonium sulphate solution, the combined acid was equal to 0·0004 equivalents per gram of protein and the proportion of acid free was 0·7 % of the total (0·0000251 N).

On addition of alkali to the original solution most was employed in decomposition of the acid protein salts, and diminution of the free acid was very gradual.

The effect of acid upon coagulation rate was studied in a series of respectively less and less acid, neutral, and faintly alkaline solutions prepared by addition of different amounts of alkali to the original acid solution.

The effect of acid upon coagulation rate is considerable. The addition to a solution of egg albumen crystals of 4 c.c. $\frac{N}{10}$ alkali per gram protein (*i.e.* the amount necessary to neutralise) reduced the reaction rate to $\frac{1}{60}$th.

The influence of acid in accelerating the coagulation rate of a neutral solution of egg-albumen is at first relatively small; with each successive addition of acid its influence becomes disproportionately greater.

The mean coagulation rate of egg-albumen is not directly proportional to the hydrogen ion concentration. The velocity of the reaction increases at first more slowly and subsequently more quickly than the hydrogen ion content. It is possible however that hydrogen ion concentration may be a factor in determining reaction rate.

As, however, most of the acid added, combines to form salts it may be that the whole or part of its effect upon reaction rate is due to such salts reacting with water more rapidly than protein itself and the more acid salts more rapidly than the less acid salts.

The free acid in a solution of egg-albumen crystals diminishes and even disappears as coagulation proceeds. The quantity of free acid fixed by the coagulation of a definite quantity of protein is at first nearly proportional to the concentration of free acid; as this concentration increases, the amount fixed falls more and more short of proportionality. The curve suggests that the phenomenon is one of adsorption by the coagulated particles, as protein already coagulated and washed absorbs free acid from solutions in which it is suspended.

Coagulation of both proteins is influenced by temperature in accordance with the Law of Arrhenius or some similar logarithmic law. The temperature coefficient is exceedingly high, viz. 1·91 per degree centigrade for egg-albumen and 1·3 per degree for hæmoglobin.

"Heat coagulation" is a reaction between protein and water and the effect of temperature is merely to accelerate it. The reaction has an extraordinarily high temperature coefficient and the velocity is influenced to a marked degree by a variety of conditions, most important among which is the presence of acid. It is improper to speak of proteins as having any particular "coagulation temperature" at which they are thrown out of solution. Notwithstanding this, the temperature, at which under precisely similar conditions a precipitate is first visible, may be useful in differentiating proteins, and can even be utilised to separate to some extent two proteins which at the same temperature are possessed of greatly different reaction rates.

The existence of an optimum temperature of the reaction, which is characteristic of many processes in which proteins in solution are concerned, *e.g.* various enzyme actions, and which has even been regarded as evidence of "vital action," distinguishing these reactions from ordinary chemical ones, (Duclaux, 1910) is capable of a simple interpretation. In the solution two processes are at work: (1) the operations under the influence of the enzyme, and (2) the destruction of the enzyme by water. If the influence of temperature upon the velocity of the enzyme destruction is greater than upon the velocity of the enzyme action, as the temperature rises, the effect of the former, negligible at first, counteracts the latter to a greater and greater extent, and a point must sooner or later be reached when the rate of destruction is so great that the enzyme action is rapidly arrested.

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Nº 11

THE FLEAS COMMON ON RATS IN DIFFERENT PARTS OF THE WORLD AND THE READINESS WITH WHICH THEY BITE MAN.

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(With Plate II.)

THE appetite for mankind of the fleas which infest rats in different parts of the world has become a question of epidemiological importance in view of the conclusion now generally accepted, that in the present pandemic these parasites play the principal part in the transmission of plague from rat to rat and from rat to man.

Whether rat fleas bite man has been much controverted, ever since Ogata (1896) and Simond (1898) put forth the theory that bubonic plague was conveyed by infected fleas. The difference of opinion is to some extent due to want of recognition of the fact that rats in different parts of the world, and also when living under varying conditions in the same part of the world, may harbour fleas belonging to different species. After making due allowance for this source of error, however, the fact remains that two observers experimenting with the same species of fleas have, not infrequently, come to opposite conclusions. It is therefore clear that the number of observations recorded and the precise conditions under which the experiments were made must be taken into account.

It is not necessary to enumerate all the species of flea which have, on occasion, been captured off rats, because this has already been done by Tiraboschi (1904), Shipley (1908) and quite recently by Rothschild (1910). From the point of view of epidemiology, it is only important to know, what fleas infest rats in different parts of the world and whether these fleas bite man with any readiness.

Fleas commonly infesting rats.

Knowledge on this point is incomplete. It is only recently that a large number of rats has been examined with this object and so far comparatively few localities have been studied. The occurrence of plague in a locality has alone provided a sufficient excuse for taking a census of the fleas infesting the rats.

As far as at present known the fleas commonly infesting both *Mus rattus* and *Mus decumanus* are:

1. *Xenopsylla cheopis* (Rothschild).
    Synon. *Pulex cheopis* Rothschild, 1903.
    Pulex pallidus Tidswell, 1903.
    Pulex brasiliensis Baker, 1904.
    Pulex murinus Tiraboschi, 1904.
    Pulex philippinensis Herzog, 1904.
    Loemopsylla cheopis (Rothsch.) Rothschild 1908.
2. *Ceratophyllus fasciatus* (Bosc.).
3. *Ceratophyllus anisus* Rothschild, 1907.
4. *Ctenopsylla musculi* (Dugès).
5. *Ctenophthalmus agyrtes* (Heller).

The dog flea (*Ctenocephalus canis*), the cat flea (*Ctenocephalus felis*), the fowl flea (*Echidnophaga gallinacea*) and the human flea (*Pulex irritans*) are also sometimes found upon rats, but they appear to be only occasional visitors. In certain localities, however, they have formed quite a considerable proportion of the fleas captured. Tiraboschi (1904, p. 259) states that in several regions of Italy as many as 25 °/₀ to 30 °/₀ of the fleas on rats were dog and cat fleas, and Verjbitski (1908, p. 165) found *Ct. felis* or *canis* on 12 °/₀ of rats (300) in Cronstadt. In Dar-es-Salam (German East Africa) *Echidnophaga gallinacea* accounted for 22 °/₀ of fleas taken off rats (Schuberg and Manteufel, 1910). As may be seen from Table I, *P. irritans* has seldom been found except in San Francisco, where it was present to the extent of 9 °/₀ of the total fleas taken.

Distribution of rat fleas.

The particular fleas found in greatest numbers upon rats seem to be determined by geographical position, including climate, and the habitat of the rats examined. The influence of species, whether *Mus decumanus* or *Mus rattus*, upon the parasite carried appears to be indirect and brought about by a general difference in the mode of life and distribution of the two species.

In Table I below we have collected all the available quantitative data upon the subject which are based upon the examination of a sufficient number of fleas to permit the facts to speak for themselves. The results are, therefore, independent of the impressions of the individual observers. For the reason mentioned above (p. 123) such data are only forthcoming from comparatively few places, but they are sufficient to draw certain broad conclusions, as to the distribution of some of the commonest parasites infesting rats.

As an instance of the effect of geographical position independent of climatic conditions, the replacement of *Ceratophyllus fasciatus* by *Ceratophyllus anisus* in Japan (Kitasato, 1909) may be mentioned, and the occurrence of *Xenopsylla scopulifer* Rothschild only in Africa. The influence of temperature will be discussed when dealing with the distribution of *Xenopsylla cheopis*.

By habitat, we mean differences in conditions and mode of life depending upon whether the rats live in the fields, sewers, docks, barns, stores, granaries or poultry houses, &c. These varying surroundings will bring the rats into association with other animals and some amount of exchange of parasites may take place. Provided these parasites can flourish upon a diet of rat-blood, they may establish themselves locally on these rodents. An instance is the prevalence of *Ctenopsylla musculi*, the common mouse-flea, upon rats living under conditions in which rats and mice come together, such as in barns, provision stores and on some ships; the presence of fowl-fleas and dog-fleas in considerable numbers upon rats inhabiting poultry farms and stables is to be explained in the same manner. The large proportion, 52 °/₀, of *Ctenophthalmus agyrtes* found by Martin and Rowland (1911) upon *Mus decumanus* living in the open in this country is another striking illustration of the case in point, the usual host of this flea being, according to Rothschild, the field vole.

(1) *Xenopsylla cheopis* (Rothschild). This flea was described by Rothschild (1903 and 1906) after examination of specimens taken from several small rodents in Egypt and the Soudan. It is now known to be the common rat flea in the tropical and sub-tropical parts of the world, occurring in some regions to the almost entire exclusion of other species. In India[1], where rat fleas were very extensively examined in four places,

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[1] The authorities for this and the following statements are given in the last column of Table I.

it formed nearly 100 % of all the rat fleas found in three instances (Table I); in the fourth (Punjab) the same was true in the warmer months, 2 % of *Ceratophyllus fasciatus* appeared in the cooler weather. In Manila, where, however, comparatively few fleas (42) were examined, all were identified as *Xenopsylla cheopis*.

In the warmer parts of the temperate regions, *e.g.* Mediterranean, Southern Japan, San Francisco and Australia as far south as Sydney, it occurs in varying proportion according to the time of year.

Xenopsylla cheopis is also stated to be common on rats in South Africa and Central and South America (Jordan and Rothschild, 1908).

One specimen has been found at Plymouth by Giles (1906) and this is the only recorded instance of its appearance in this country. We have no doubt, however, that it would be frequently found if, during September, a sufficiently extensive examination were made of rats from ships in English ports[1].

The extensive census of rat fleas in various parts of India made throughout the year by the Plague Commission (1908, p. 266, 1910, pp. 460 and 524) shows a striking maximum for flea prevalence (*X. cheopis*) during some portion of the year, the mean number infesting rats increasing three to ten fold.

In those temperate climates (Japan, Sydney, Marseilles) where observations have been directed to the point, a seasonal prevalence of these fleas has also been observed, the numbers increasing greatly both actually and relatively towards the end of summer and declining with the onset of cold weather (Kitasato, 1909; Tidswell, 1910, p. 20; Gauthier and Raybaud, 1910, II.).

(2) *Ceratophyllus fasciatus* (Bosc.). This species is, according to Rothschild (1906, p. 483) and Tiraboschi (1907, p. 595), the common flea upon *Mus decumanus* and *Mus rattus* in this country and in the northern and central parts of Europe. Galli Valerio (1900, I.), however, found it rarely in Lausanne. He believes that *Ctenopsylla musculi* is the usual flea on rats in this city and states that it is commonly present in Holland and at Halle and that he himself found it at Milan.

Recently Martin and Rowland (1911) found it to constitute about one half (48 %) of 1057 fleas taken from *Mus decumanus* in Suffolk and Elstree (Herts.).

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[1] Since this paper was written Dr Boycott has taken two specimens of *X. cheopis* off *Mus decumanus* captured at Guy's Hospital, London, S.E. The specimens were identified by Mr Rothschild.

TABLE I.

				Percentage of	
Locality	Source	Species	No. of rat fleas examined	*Xenopsylla cheopis*	*Xenopsylla scopulifer*
India: Bombay ...	Houses, gullies outbuildings	*M. rattus* and *M. decumanus*	216,000	100	—
Poona	Houses, out-buildings	*M. rattus*	240,000	100	4 only
Belgaum ...	Houses	,,	380,600	100	—
Punjab... ...	Houses	,,	54,000	98	—
Australia: Brisbane ...	—	*M. rattus* and *M. decumanus*	103	76·7	—
Sydney ...	—	,, ,,	100	81·0	—
,, ...	—	—	1926	61·8	—
San Francisco ...	—	*M. rattus* and *M. decumanus*	10,972	21·4	—
,,	—	,, ,,	15,554†	32·1	—
,, (Oakland)...	—	*M. decumanus*	4140	23·2	—
Manila	—	*M. rattus* and *M. decumanus*	42	100	—
Africa: Dar-es-Salam ...	—	,, ,,	258	66·6	10·9
Japan: Yokohama, Tokio, and other large towns (winter)	—	—	2713	6·1	—
Yokohama ...	Ships	*M. rattus*	—	100	—
Awaji Island near Kobe (autumn)	—	—	835	45·5	—
,, ,, (winter)	—	—	4617	20·2	—
Marseilles	Ships	*M. rattus*	250	25·6	—
,, ...	Town	*M. decumanus*	52	5·8	—
,, ...	Ships	*M. rattus*	2276	92·7	—
,, ...	Quays	*M. decumanus*	2424	33·0	—
,, ...	Town	,,	4377	50·4	—
Tunis	—	*M. rattus* and *M. decumanus*	1242	83·6	—
Algeria: Algiers ...	—	,, ,,	2410	59·7	—
Constantine and Oran	—	,, ,,	428	35·9	—
Great Britain: Suffolk ...	Rats in the open	*M. decumanus*	584	—	—
,, ...	Rats' nests	,,	215	—	—
Herts. ...	,,	,,	258	—	—

* In some cases the fleas are not described by these authorities in accordance with the latest nomenclature; there is, however, seldom any doubt as to the species intended.

TABLE I (*continued*).

total fleas belonging to the various species*						
Ceratophyllus fasciatus	*Ctenopsylla musculi*	*Ctenophthalmus agyrtes*	*Ctenocephalus felis* and *Ct. canis*	*Echidnophaga gallinacea*	*Pulex irritans*	Observer
—	—	—	—	—	—	*Commission for Investigation of Plague in India Report*, 1907, p. 412; 1908, p. 297.
—	—	—	2 only	—	—	*Ibid.* 1910, p. 524.
—	—	—	—	—	—	*Ibid.* 1910, p. 461.
2 (winter only)	—	—	—	—	—	*Ibid.* 1907, p. 914.
—	17·5	—	5·8	—	—	Tidswell, 1903, p. 71.
10	8·0	—	1·0	—	—	,, ,,
10·9	26·7	—	0·5	—	0·05	,, 1910, p. 20.
68·1	4·5	—	0·5	—	5·6	Surgeon Rupert Blue, private communication, 1908.
52·0	4·6	—	1·8	—	9·4	Carroll Fox, 1910.
44·8	31·6	—	0·2	—	0·2	,, ,,
—	—	—	—	—	—	Herzog, 1905.
—	—	—	—	22·5	—	Schuberg and Manteufel, 1910.
66·5‡	27·4	—	0·3	—	—	Kitasato, 1909.
—	—	—	—	—	—	,, ,,
58·6‡	25·5	—	3·9	—	—	,, ,,
28·1‡	20·7	—	0·03	—	0·1	,, ,,
2·4	71·2	—	—	—	0·8	Gauthier and Raybaud, 1903.
86·5	3·8	—	3·8	—	—	,, ,, ,,
6·7	0·5	—	0·1	—	—	,, ,, 1910.
51·5	10·2	—	5·2	—	—	,, ,, ,,
35·1	7·9	—	6·4	—	—	,, ,, ,,
5·1	10·1	—	0·4	—	0·1	Conseil, 1909.
13·5	20·1	—	6·3	—	0·4	Raynaud, 1909.
22·9	36·9	—	4·3	—	—	Billet, 1908.
55·5	—	44·4	—	—	—	Martin and Rowland, 1911.
47·0	—	53·0	—	—	—	,, ,, ,,
33·3	—	66·7	—	—	—	,, ,, ,,

† It is possible that some of these fleas may be included among those previously reported upon by Surgeon Blue.

‡ These numbers refer to *Ceratophyllus anisus*.

In warmer temperate regions *Ceratophyllus fasciatus* together with *Xenopsylla cheopis* forms the bulk of the rat-flea population, *e.g.* San Francisco, where out of a total of 10,972 rat fleas collected, 68 °/₀ were *Ceratophyllus fasciatus* and 21·4 °/₀ *Xenopsylla cheopis.* As a rule, in such climates, the proportion of the former increases relatively during the winter, and that of the latter during summer.

Ceratophyllus fasciatus has not been found in the warmer parts of India, but in the Punjab it appears during the cooler half of the year, November to March, and accounts for 2 °/₀ of the total.

In the Himalayas (Simla) this flea is common.

(3) *Ceratophyllus anisus* Rothschild. In Japan this flea appears to occupy the position assumed by *Ceratophyllus fasciatus* elsewhere (Kitasato, 1909), occurring to a relatively larger extent in the winter than in the summer, when *Xenopsylla cheopis* makes its appearance in some places in large proportion.

(4) *Ctenopsylla musculi* (Dugès). This is the flea which usually infests the house mouse, *Mus musculus.* Tiraboschi (1907, p. 60) found it on rats in all parts of Italy, but less frequently on *Mus decumanus* than on *Mus rattus.* According to Verjbitski (1904) it is the flea most common upon rats in Cronstadt.

As may be seen from Table I, this flea furnishes a considerable contribution to the total fleas harboured by rats in many widely separated parts of the world: North America, Europe, Japan, Australia. It was rarely found in India.

It is sometimes very prevalent upon *Mus rattus* from ships; on one occasion at Marseilles, among 250 fleas from this source Gauthier and Raybaud (1903) identified 178 as *Ctenopsylla musculi.*

(5) *Ctenophthalmus agyrtes* (Heller). According to Rothschild, this flea is common in England and Europe upon field mice and bank voles and occurs also on *Mus decumanus* when captured in the open. The recent observations of Martin and Rowland (1911) on the fleas from rats living on farms in Suffolk, Hertfordshire and Hampshire showed an unexpected proportion of this flea to be present (Table I). The rats examined were living in burrows in the fields or in corn stacks. It appears therefore that in these localities *Ctenophthalmus agyrtes* accounts for about half of the fleas infesting rats living under the conditions mentioned. Whether the same obtains in other rural parts of England is at present unknown.

Members of the above five species constitute the bulk of fleas, which up to the present have been shown to commonly occur upon rats.

One other flea, *Xenopsylla scopulifer* Rothschild, which, according to Schuberg and Manteufel (1910), replaces *Xenopsylla cheopis* to some extent in German East Africa, demands mention. It was described by Rothschild (1905) and obtained off *Saccostomus campestris* and *Mus auricomis* in Zululand. It is closely allied to *Xenopsylla cheopis*.

The readiness with which various rat fleas attack man.

1. *Xenopsylla cheopis.* That this flea readily feeds on man was observed by Tidswell (1903) and Gauthier and Raybaud (1903). This was confirmed by Liston (1904), who also collected many observations showing that, in the absence of its natural host the rat, *X. cheopis* is attracted to mankind. This fact was subsequently confirmed by the Commission for Investigation of Plague in India (*Report*, 1907, p. 472 and 1908, p. 249).

2. *Ceratophyllus fasciatus.* With regard to the readiness with which this flea attacks man considerable divergence of opinion has hitherto existed. According to Wagner (quoted by Tiraboschi 1904, p. 180), Tiraboschi (1904, p. 266) and Galli-Valerio (1907) this flea does not bite man. On the other hand, Gauthier and Raybaud (1903 and 1909) and McCoy and Mitzmain (1909) found that when hungry it fed on man with readiness.

We have made some hundreds of experiments on this question and are at a loss to understand the negative conclusion arrived at by Tiraboschi and Galli-Valerio.

The fleas used in our observations had been bred in the Institute and were kindly placed at our disposal by Professor E. A. Minchin. The fleas were caught in the breeding cages and placed separately in test tubes, which were kept in tins or jars in a dark cupboard covered with damp cloths to prevent undue drying of the atmosphere.

The fleas were starved for periods varying from 24 hours to 14 days before testing their inclination to feed on man. The tubes were inverted upon the naked skin of the arm of the subject and the flea carefully watched for a period of two minutes. If it did not bite during this time it was regarded as negative for our experiments. Frequently the flea attached itself instantly or within a few seconds and began to feed without delay; sometimes it took a little longer to get started, but usually if it were going to bite at all it did so before one minute had elapsed.

As soon as the flea attached itself the tube was removed and the behaviour of the insect was watched with a hand-lens. The position assumed was generally characteristic. After a preliminary investigation of the surface with the tip of the labium and the maxillary palps, the insect was seen to press its head firmly on to the skin and raise its abdomen steeply into the air. The mandibles and epipharynx, which constitute the "pricker" and enter the skin, are too small to be seen with a magnification of 10 to 20 diameters but the labium in which these parts are carried, can be clearly observed. As the "pricker" is pressed into the skin the slack of the bifurcated labium is either bent in the form of two bows or flexed as the fingers are in making a rest for a billiard cue. The insect is so much absorbed in the occupation of sucking, that it can be rotated 180 degrees around its pricker as axis without being detached. In a few cases in which, for some reason or other, notwithstanding the manifest efforts of the animal, no blood entered the stomach, this attachment of the insect was regarded as evidence of biting for statistical purposes. Usually, however, there was no difficulty in seeing the blood entering the stomach in rapid jets. A pink flush spreads over the anterior portion of the abdomen, which gradually reddens as the stomach becomes distended.

The fleas which did not bite usually wandered about during the two minutes they were under observation, and in some cases showed evident dislike, making efforts to crawl or jump back into the tube, away from the skin of the subject.

The results of 517 experiments with 364 fleas and 8 persons are given in Table II. The proportion of fleas which bit varied from 36 °/₀ to 77 °/₀, giving a mean value for man of 59·6 °/₀. The variation between different people is large enough to warrant the conclusion that the suggested idiosyncrasy is a reality.

In order to obtain a satisfactory control for these experiments, a similar set were undertaken, using the rabbit and the rat as subjects; the results are also given in Table II. The experiments were in all details similar to the above, except for the expedients adopted for keeping the animal quiet during the observations.

The rabbit was rolled up in a cloth, and remained motionless on a table in a sleepy condition. The fleas were each allowed to remain for 2 minutes on the inside of the ear. 32 fleas were tried of which 23 bit, a proportion of 72 °/₀.

The rat was secured by a similar method. Two bandages were used, one for the neck and shoulders, and one for the thighs and legs, leaving a portion of the abdominal wall free. The area of skin upon which the fleas were tried was shaved. The rat was laid on its back, the head comfortably supported with a pillow of cotton wool, and kept from rolling away by fastening the ends of the bandages to a board with drawing pins. The rat remained perfectly quiet and there was no difficulty in carrying out the observations. 101 fleas were tried, of which 59 bit.

Under the conditions of these experiments *Ceratophyllus fasciatus* fed upon man as readily as upon a rat.

TABLE II.

Ceratophyllus fasciatus.

Subject:—	C.J.M.	H.C.	A.M.	J.H.S.	H.Y.	G.F.P.	H.W.A.	S.R.	Summary	Rat	Rabbit
Total No. of Exps.	161	118	39	33	52	51	40	23	517	101	32
Positive... ...	106	65	30	12	36	25	19	15	308	59	23
Negative ...	55	53	9	21	16	26	21	8	209	42	9
°/₀ positive ...	65·8	55·1	76·9	36·4	69·2	49·0	47·5	65·2	59·6	58·4	71·9

Cage experiments with Ceratophyllus fasciatus.

The readiness with which *C. fasciatus*, if hungry, will attach itself to man was demonstrated in the following manner. The rat was removed from a flea breeding-cage, into which 100 fleas had been placed a few days previously. Four days later, the hand and arm of a number of persons were, at different times during the day, placed in the cage and allowed to remain there for two minutes. The results are given in Table III, where it is seen that in one case as many as 18 fleas jumped upon the arm, many of which could be felt at once to bite vigorously.

TABLE III.

Cage experiment 16. XI. 10.

Observer:—	C. J. M.			H. C.	A. M.		J. L. C.	H.Y.	G.F.P.	H.A.	H.B.
	1	2	3		1	2					
No. of fleas found upon arm after 2 mins.	7	3	7	18	3	3	3	6	0	6	14

Further observations to test this point were made by putting the arm into cages in which the fleas were bred. These cages consisted of two compartments, separated from one another by coarse-meshed wire netting. The rat inhabited one compartment, and the other served as breeding ground for the fleas which could freely pass from one to the other. When the hand was placed in the compartment containing the rat the result was negative, but when inserted into the other, although within six inches of the rat, numbers of fleas (in one case 40) hopped on to the arm, and most of them fed immediately.

The details of these experiments are set out in Table IV below.

TABLE IV.

Cage experiment 26. XI. 10.

Observer	Cage No.	No. of fleas attaching themselves in 1 minute, outside rat compartment but within 6 inches of rat
C. J. M.	1	8
C. J. M.	2	40
G. K.	2	20

The bite of *Ceratophyllus fasciatus* was not, as a rule, followed by either irritation or swelling. In the case of one individual, however, the results were very distinct and lasted for several days. The photograph reproduced in Pl. II was taken 48 hours after 30 fleas of this species had been placed upon the arm. Every bite was followed by an inflamed papule surrounded by oedema. The irritation was considerable and lasted for some days.

3. *Ctenopsylla musculi.* According to Galli-Valerio (1900, *a* and *b*), Tidswell (1903) and Tiraboschi (1904, p. 282) this flea does not bite man. Verjbitski (1908, p. 164) came to the same conclusion. This observer made 40 experiments on himself, after starving the fleas for different periods, but in no case did they feed. On the other hand, McCoy and Mitzmain (1909), and Gauthier and Raybaud (1910) found that this flea would occasionally bite man.

McCoy and Mitzmain's observations were made with 15 fleas. Each flea was tested on successive days for five minutes until it either died or escaped. With these opportunities eight of them were induced to feed on at least one occasion.

In our own experiments the fleas were bred in the Institute in cages with mice. From a cage started in December 1910, enough fleas were obtained on January 14, 1911, for a satisfactory experiment, viz.: 71 fleas. On the day following their removal from the cage only 46 were still alive. This species would appear to be less robust than either *Ceratophyllus fasciatus* or *Ctenophthalmus agyrtes*, a fact also noted by McCoy and Mitzmain.

With these 46 fleas, starved for 24 hours, the 111 experiments set forth in Table V were made; 4 of the fleas bit and remained attached for several minutes, in fact until removed. In only one of these was blood found at the subsequent examination. 11 fleas which had given negative results were placed upon the shaved abdomen of a mouse; 9 of them fed. The same conditions obtained as in the case of *Ceratophyllus fasciatus* and the fleas were allowed to remain on the skin for two minutes only.

TABLE V.

Experiments with Ctenopsylla musculi, 14. I. 11.

Subject	H. C.	C. J. M.	S. R.	Summary	Mouse
No. of Experiments	36	47	28	111	11
Positive ...	1	1	2	4	9
Negative ...	35	46	26	107	2
°/₀ positive ...	—	—	—	3·6	81·8

Our results are in agreement with those of McCoy and Mitzmain and Gauthier and Raybaud. This flea will occasionally bite man, but it evinces small inclination to do so.

4. *Ctenophthalmus agyrtes.* No observations to determine whether this species bites man have, as far as we know, been previously made.

The fleas for our experiments were obtained from rats and rats' nests examined during the investigation of plague among rats in Suffolk (November 1910 to January 1911) by Martin and Rowland (1911).

In all 98 experiments were made with 68 fleas and four persons (C. J. M., H. C., S. R. and A. M.); in every case the result was negative.

As control, some of the fleas, which would not feed upon man, were tested upon a rat. Unfortunately we omitted to do this until our supply was nearly exhausted and the number of fleas constituting the control was only 19. These 19 were placed upon the arm and allowed to remain for two minutes with negative result. Immediately afterwards they were placed upon the shaved skin of a rat (in the manner described above) under precisely similar circumstances as those in which they had failed to bite us. 11 fastened upon the rat and fed.

We have not yet succeeded in breeding a supply of *Ctenophthalmus agyrtes*, so that our experiments are not so numerous as with *Ceratophyllus fasciatus*, but they show that the former flea exhibits no inclination to feed upon man.

The following experiment described by Rowland and one of us (1911) illustrates the difference between *Ceratophyllus fasciatus* and *Ctenophthalmus agyrtes* as regards their appetite for man. A bottle containing 23 unidentified fleas, taken from wild rats three days previously, was inverted upon the arm of one of the authors (S. R.). Eleven attached themselves and remained feeding; the remainder, 12, were shaken back into the bottle. The 11 which had fed were chloroformed and on examination were identified as *Ceratophyllus fasciatus*. The remaining 12 were placed upon C. J. M.; one fed and this also was identified as *Ceratophyllus fasciatus*. The 11, which had bitten neither observer, were found to consist of eight *Ctenophthalmus agyrtes* and three *Ceratophyllus fasciatus*.

SUMMARY.

(1) *As far as is at present known*, the great majority of the fleas infesting *Mus rattus* and *Mus decumanus* in different parts of the world, belong to either the species *Xenopsylla cheopis*, *Ceratophyllus fasciatus*, *Ceratophyllus anisus*, *Ctenopsylla musculi* or *Ctenophthalmus agyrtes* or are comprised of some admixture of these five species.

(2) *Xenopsylla cheopis* is the most prevalent in the tropics and sub-tropical regions and often occurs there to the almost complete exclusion of other species. It is common during summer and autumn in some of the warmer parts of the temperate zone, more especially in ports which have maritime intercourse with the tropics.

(3) In the cooler regions *Ceratophyllus fasciatus* is the most universally distributed flea and is associated with more or less of *Ctenopsylla musculi* and *Ctenophthalmus agyrtes* according to the locality and the habitat of the particular rats.

(4) In Japan *Ceratophyllus fasciatus* is replaced by *Ceratophyllus anisus*, a closely allied species.

(5) The numerous other fleas which have been captured off rats are only occasional visitors.

(6) *Ceratophyllus fasciatus*, like *Xenopsylla cheopis*, readily bites man. Out of 517 experiments 308 fed, or 59 % were positive. In 101 experiments, under identical circumstances with a rat, 59, or 58·4 % of the fleas fed.

(7) The experiments with *Ceratophyllus fasciatus* were made upon eight persons and evidence was obtained of preference on the part of the insects for particular individuals.

(8) 111 experiments were made with 46 specimens of *Ctenopsylla musculi*; only 4 fed = 3·6 %, whereas 9 out of 11 fed on a mouse.

(9) 68 specimens of *Ctenophthalmus agyrtes* were tried, in some cases upon three persons. None fed, whereas 11 out of 19 of the same fleas fed on a rat under identical conditions.

GENERAL CONCLUSION.

Xenopsylla cheopis and *Ceratophyllus fasciatus* are the species of rat fleas which, when hungry, readily bite man. There is no reason to suppose that, other things being equal, *Ceratophyllus fasciatus* would not be as efficient an agent in the transmission of plague from rat to man as *Xenopsylla cheopis* has been shown to be in India.

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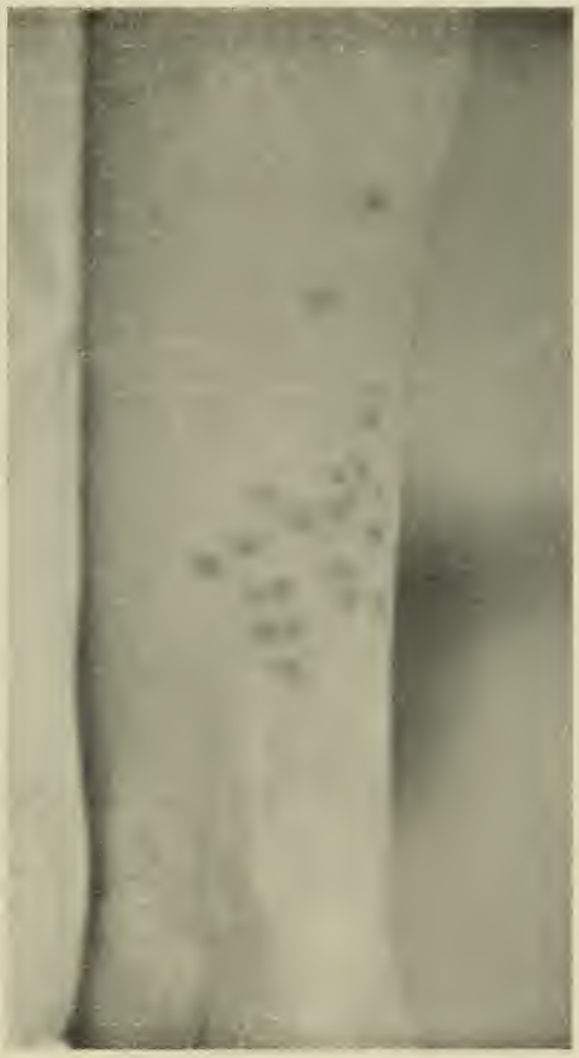

Forearm of A. M. 48 hours after 30 "Ceratophyllus fasciatus" had been placed upon it, 24 of the fleas having fed.

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An Examination of the Blood Serum of Idiots by the Wassermann Reaction.[1]

By H. R. DEAN, M.B.

THE examination of the blood serum of idiots by the Wassermann reaction has been the subject of several papers. Raviart, Breton, Petit, Gayet, and Cannac [6] examined 246 cases, of which 76 were found to give a positive reaction. Kellner, Clemenz, Bruckner, and Rautenberg [2] examined 216 cases, of which 13 gave a positive reaction by Stern's method and 9 gave a positive reaction by the original Wassermann method. To the 13 cases must be added 3 cases which were deficient in complement and were found to be positive by the original method. H. Lippmann [4], working in Wassermann's laboratory, examined 78 cases at Uchtspring, and obtained a positive reaction in 7 cases—that is to say, 9 per cent. An examination of the cases at the Dalldorf Asylum gave a result of 13·2 per cent. Lippmann also examined 77 cases by clinical methods, and decided that 40·2 per cent. showed signs of congenital syphilis.

At the suggestion of Professor Wassermann, I investigated the inmates of the Wilhelmstift, an asylum for idiots at Potsdam. The inmates were all children or young adults, and the majority were cases of simple idiocy or imbecility. The method was as follows: (1) All the cases were examined for evidence of congenital syphilis; (2) a sample of blood was taken from the arm vein and tested by the Wassermann reaction; (3) subsequently the cases which had given a positive reaction were submitted to a further clinical examination. The serum test was carried out strictly in accordance with the original method, and watery extract of congenital syphilitic liver was used as antigen in all cases. The extracts and the hæmolytic system were carefully standardized by titration, and in the actual test adequate controls from well-authenticated cases were invariably employed. No test was considered positive when more than the slightest trace of hæmolysis could be detected; 330 cases were examined, of which 51 gave a positive reaction—that is to say, 15·4 per cent. Among the 51 cases which gave a positive serum reaction, 7 were found which had definite signs of syphilis, and 3 or 4 in which syphilis might have been suspected but not with certainty diagnosed. There were 2 cases with definite signs, which gave a negative reaction. That is to say, among the 330 patients were 9, or including the doubtful cases 13, which from physical signs and symptoms would have justified the diagnosis of syphilis. The results in detail were as follows:—

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[1] From the Serological Department of the Royal Institute for Infective Diseases in Berlin.

	Cases examined	Positive
Simple idiocy of all grades	287	44
Congenital spastic diplegia (Little's disease)	15	1
Marked hydrocephalus	14	4
Epilepsy	1	1
Microcephalic cases	4	—
Mongols	1	—
Deaf and dumb	7	1
Progressive muscular dystrophy, with mental symptoms	1	—

All the cases which gave a positive serum reaction were subsequently very carefully examined with the object (1) of discovering any sign of syphilis, which had been previously overlooked, and (2) of detecting any symptom or group of symptoms common to all the positive cases. Of the positive cases, one was subject to epileptiform convulsions and showed slight choreic movements, one had strabismus and nystagmus, one had a right-sided hemiplegia, one had spastic diplegia and conformed to the type of Little's disease, one was a deaf-mute, two were aphasic. Among the remaining cases I was unable to detect any evidence of a local lesion.

An examination was made of the cerebrospinal fluid from 12 cases, which had given a positive serum reaction. In only one case was a positive reaction obtained. I also obtained for examination specimens of serum from the parents of 10 of the positive cases. The results were as follows:—

Initial of patient's name	Age of patient	Result of examination of parents' serum
B.	9	Father positive Mother ,,
Har.	15	,, ,,
Kr.	11	Father ,, Mother ,,
M.	11	Father ,,
V.	13	Mother ,,
O.	11	Father negative
Sc.	16	,, positive
R.	12	,, negative
N.	9	,, ,, Mother ,,
He.	14	,, positive

Thus among 13 parents of children giving a positive reaction, 9 were found to give a positive reaction.

It will be noticed that in the above table in the case of the patient Har. a positive reaction was obtained at an interval of fifteen, and in the case of Sc. at an interval of sixteen, years after the birth of a syphilitic child. The period during which a positive reaction may be obtained is known to be extremely variable in the case of the acquired form of the disease. In the congenital form it might be expected that the percentage of positive results would bear a close relation to the age of the patients examined. A grouping of the 330 cases according to age gives the following result:—

	Examined	Positive	Percentage of positive results
(I) Patients aged 10 and under (of these two only were less than 5 years old)	94	20	21·27
(II) Patients from 11 up to 15 years of age inclusive	142	24	16·9
(III) Patients from 16 to 20 years of age	66	4	6·06

Of patients aged from 21 to 30, 24 were examined with 3 positive results. The remaining 8 patients ranged in age from 31 to 44, and all 8 gave a negative reaction.

The above table appears to show that the percentage of positive results diminishes rapidly after the sixteenth year, and that a larger percentage of positive results might be expected from the examination of a series of very young cases. In any case, the average age of the patients investigated must be regarded as an important factor in any estimation of the prevalence of congenital syphilis, and it seems to me possible that the very contradictory results already published may be reconciled by taking the age factor into consideration. Of the 51 cases in which a positive serum reaction was obtained, 7 only showed conclusive evidence of congenital syphilis from a clinical standpoint. In the remaining 44 cases a diagnosis of syphilis rested on the evidence of the serum test.

At the present time it is, I think, generally admitted that a positive result obtained by the Wassermann reaction affords strong evidence of a syphilitic infection, and it is superfluous for me to cite evidence in support of its reliability. The practical value of the test must necessarily be measured by the number of those cases in which the positive serum reaction is the only obtainable sign of a syphilitic infection. In cases where latent syphilis is suspected, a single negative result affords little information, but the value of a positive result has been established beyond doubt. In this connexion great interest has been aroused by the cases reported by Plaut and others, in which the husband or wife of a syphilitic has been shown to give a positive reaction, but has never shown any sign or symptom of the disease. It is even held that the serum reaction may be the only sign from first to last of a syphilitic infection. Linser [3], quoted by Bruck [1], has examined a series of children of syphilitic patients, and finds that two-thirds give a positive serum reaction, while only one-third show any other sign of infection. Numerous cases are also on record in which it has been demonstrated that the apparently healthy mothers of syphilitic children give a positive reaction, and, conversely, the serum test has been positive in apparently healthy children of syphilitic mothers. The numerous investigations on these lines tend to prove that the evidence of a positive serum reaction may be accepted even in the absence of the usual signs and symptoms of the disease. If, however, it is granted that the 51 cases of this series were the subjects of congenital syphilis, or at least the children of syphilitic parents, it still remains open to question whether there is any evidence of a causal relation between syphilis and idiocy. It might very reasonably be held that the two conditions were unconnected, or that syphilis effected a very remote influence, as a predisposing cause, by in some way impairing the vitality of the parent or offspring. It can also be maintained that a high percentage of congenital syphilis is to be expected among the children of parents whose mental and moral faculties are presumably below the average level.

I had hoped that by a careful examination of those cases which had given a positive result to the serum test it might be possible to detect some symptom or group of symptoms which was common to all. This I failed to do. Very few of the positive cases showed any evidence of a gross lesion in the central nervous system; and this, I think, is quite in accordance with what one might expect, for the gross changes in the brain which are known to be due to congenital syphilis are not compatible, as a rule, with a continuance of life. If a causal relation exists between congenital syphilis and idiocy, the condition which arises may perhaps be classed as parasyphilitic. The absence of the ordinary signs of congenital syphilis in idiocy is closely paralleled in the already authenticated parasyphilitic diseases. It is, of course, notorious that tabes and general paralysis commonly occur in patients where the early symptoms of syphilis have been mild or even unnoticed. Among the cases of the juvenile form of general paralysis collected by Mott [5] quite half were found to show no sign of congenital syphilis, but nevertheless to have been born of syphilitic parents and to have brothers and sisters who exhibited the ordinary signs of the disease.

It seems to me reasonable to think that many cases of idiocy should be classed with that form of syphilis which manifests itself alone by a selective toxic action on the elements of the central nervous system. I do not wish to attach an exaggerated importance to the results of the examination of the serum in one series of cases, but when it can be shown that a considerable percentage of idiots afford evidence of a syphilitic infection, and since it is well known that the virus of syphilis is capable of exercising a selective action on the central nervous system in cases in which there is no other evidence of the disease, I think it is not unreasonable to infer a causal relation between the two conditions.

From the therapeutical standpoint it is hardly, I think, to be expected that any improvement will be obtained by the mercurial treatment of cases of established idiocy, which give a positive Wassermann reaction. On the other hand, it does not seem unreasonable to suggest that the information which can be gained from the Wassermann reaction should be made of use as part of a system of prophylactic treatment. It should not be impracticable or even very difficult to secure a specimen of the blood serum of any woman during pregnancy, in cases in which any suspicion of syphilis might be entertained. If a positive reaction was obtained it can hardly be doubted that the active treatment of the mother, followed later by treatment of the infant, would be the means of averting or modifying the occurrence of symptoms. It has been even suggested by Professor Wassermann that a routine examination should be undertaken of the blood serum of every patient who is admitted to a lying-in hospital. The proposal is the logical result of the belief that a syphilitic infection may exist in an apparently healthy subject, and that a reliable diagnosis can be obtained by the employment of the Wassermann reaction.

In conclusion, I have pleasure in expressing my gratitude to Professor Wassermann for the kind interest which he took in these experiments. My best thanks are also due to the Director and Medical Officers of the Wilhelmstift Asylum in Potsdam for their kindness in assisting me in collecting samples of serum and in the examination of the patients.

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COMPARISON OF THE ORIGINAL WASSERMANN METHOD WITH SOME OF ITS MODIFICATIONS.

Contribution to a Discussion in the Sections of Pathology and Bacteriology at the Annual Meeting of the British Medical Association, London, July, 1910.

By H. R. DEAN,
Lister Institute, London.

Relative Value of Different Methods.

One of the most interesting as well as the most difficult questions in connexion with the Wassermann reaction is that which deals with the relative value of the various methods of preparing the extract. So long as we remain ignorant of the actual nature of the antigen it seems hardly possible to deal with the question from a quantitative standpoint.

Supposing that we compare two extracts—the one made from the liver of a congenital syphilitic, the other from a normal guinea-pig's heart, we are not so much comparing the methods of preparation as the relative richness in antigen of two chance specimens. One guinea-pig's heart will differ considerably from another, and no two specimens of liver will be found to be equally rich in antigen. It seems to me impossible at the present time to make a quantitative comparison between extracts of normal and syphilitic organs. On the other hand, it is possible to draw some sort of conclusion by testing a number of serums against two extracts and noting the results.

I have records of 195 cases in which I have compared an alcoholic extract of normal guinea-pig's heart and a saline extract of syphilitic liver. Of these 195 cases a positive result was obtained with the liver extract in 31 cases and with the extract made from normal guinea-pig's heart in 22 cases. In 1 case only was a positive result obtained with the alcoholic, but not with the watery extract. The extracts used in these experiments had been carefully tested and found to be good; that is to say, they had been found to give a positive reaction in very small doses when titrated with test serums from undoubted cases of secondary syphilis.

The majority of the 195 cases which I have quoted formed part of a series of cases of children who were

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From the Bacteriological Department of the Lister Institute.

examined with a view to establishing a diagnosis of congenital syphilis, and the large majority of the cases which gave a positive result showed no other evidence of syphilis. On the other hand, I have never been able to demonstrate any difference between extracts made from normal and syphilitic organs in testing serums from cases of obvious secondary and tertiary syphilis. The class of case in which I have found a difference in the result between the extracts made from normal and syphilitic organs has unfortunately been that in which the serum diagnosis could not be confirmed in a satisfactory manner by clinical findings. Although I am inclined to the opinion that the extract made from syphilitic material is a more delicate reagent, it is not possible to exclude the view that it is actually too delicate On the whole, I am inclined to think that in cases of congenital syphilis, and in those cases in which many years have elapsed since the supposed infection, the watery extract of syphilitic liver is greatly to be preferred The question is very important from a practical standpoint, because it is only in latent and obscure cases that there is any real necessity for the serum reaction as an aid to diagnosis.

Another very important point with regard to the extract is its titration and standardization. It is absolutely essential to titrate a new extract in falling doses against several authenticated positive and negative serums. Extracts vary very considerably in strength and properties, and it is not safe to use a fixed dose of a fixed dilution without ascertaining if it is appropriate to the particular extract in use. It is necessary to determine the dose in which an extract without addition of serum will absorb complement, the dose in which complement is absorbed in the presence of normal serum, and the dose which, in the presence of positive serum, will bind the complement. A good extract is one which shows a large difference in the dose necessary for the fixation of complement in the presence of syphilitic and normal serum. An extract dose should be selected which can be relied on to give a positive reaction with a syphilitic serum, and which at the same time is less than half the amount which binds complement in the presence of normal serum.

Extracts should be tested before coming into use, and at fairly frequent intervals, to guard against deterioration in the qualities of the extract, which deterioration is in the case of the watery extracts often very unpleasantly sudden. Adequate standardization of the extract can, I think, be only carried out by the use of a haemolytic system which can itself be standardized, that is to say, there must be added to each tube a known definite amount of haemolytic serum and a known amount of complement. A titration which relied on the normal amboceptor and normal complement present in fresh human serum would afford little evidence which would be of service in performing experiments with other serums.

One of the great objections to all modifications which make use of the normal complement is that the worker can form no opinion of the power of the particular dose of extract to absorb complement in the presence of normal serum. In several of the modifications-commonly used reliance is placed on the normal amboceptor for sheep's corpuscles which is present in human blood. I examined 50 serums to ascertain their content of normal haemolytic amboceptor for sheep cells.

The tubes were made up to a bulk of 2 5 c.cm., and contained 0.5 c.cm. of a 1 in 20 suspension of sheep's corpuscles, and 0.5 c.cm. of a 1 in 10 dilution of fresh guinea-pig serum. The tubes were incubated for two hours at 37° C. The human serums were inactivated and used in various doses. Of the 50 serums haemolytic amboceptor was absent in 2 cases; in 7 cases haemolysis was incomplete with a dose of 0.1 c cm.—that is to say, the dose usually employed in the performance of the serum reaction for syphilis. In 4 cases complete haemolysis was produced by 0.1 c.cm serum; in 9 cases complete haemolysis was produced by 0.05 c.cm. serum; in 9 cases complete haemolysis was produced by 0.025 c cm. serum; in 17 cases complete haemolysis was produced by 0.0125 c.cm. serum; in 1 case complete haemolysis was produced by 0.005 c.cm. serum.

Thus 0 1 c.cm serum was found to contain in one case twenty minimal haemolytic doses, in 17 cases eight doses, in 9 cases four doses, in 9 cases two doses, in 4 cases one dose, in 7 cases less than one dose, and in 2 cases no haemolysis occurred.

The amount of normal haemolytic amboceptor is a variable factor, even in the original method; but in the original method the only question which arises relates to possible presence of excess of amboceptor. In the case which I mentioned in which 0.1 c cm. of serum contained twenty minimal haemolytic doses, a marked positive result was obtained without any particular precautions being observed.

To further test the effect of excess of haemolysin on the reaction, the following procedure was employed:

Several serums, known to give a positive reaction, were absorbed with sheep's corpuscles in the cold. The normal amboceptor for sheep's corpuscles was in this way removed, and its removal was proved by experiment.

The treated serums (from which the haemolytic amboceptor had been removed) were then titrated with falling doses of extract, and a similar series of tubes were put up containing similar amounts of extract and of the untreated serums. It was found that the intensity of the reaction had been but little influenced by the removal of the normal amboceptor—that is to say, the untreated serum gave almost as marked a reaction as the serum from which normal amboceptor had been removed. A sufficient dose of a haemolytic serum (rabbit v. sheep) was, of course, added to each series of tubes.

The result indicates that a positive reaction is in no great danger of being masked by excess of the normal amboceptor.

In Bauer's and Hecht's method reliance is placed on the normally present amboceptor. The danger lies, I think, not in the few cases in which the amboceptor is completely absent, but in those cases where just sufficient is present in the control tube, which contains serum alone, to produce complete haemolysis. In those cases the presence of

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extract will just upset the balance, and by the absorption of a certain amount of complement a positive result will be simulated. The presence of a known normal and a known syphilitic serum with extract does not afford adequate controls, for the control negative serum may have an unusually powerful haemolytic system

The normal complement is not so variable; nevertheless, considerable variations do occur. In an examination of 90 serums I found haemolytic complement apparently absent in 2 cases, and in 3 cases the amount present was very small. I examined 87 serums by Wassermann's method, and by the method of M. Stern. Of these, 14 were positive by the original method, and 23 by Stern's method. The 87 serums were also tested for presence and amount of haemolytic complement. Of the 9 cases which were positive by Stern's method and negative by Wassermann's method, 4 could be shown to be unusually deficient in complement.

Another factor in producing a relatively high percentage of positive results is probably the use of unheated serum. Sachs and Boas have shown that the serum of an undoubted syphilitic will give a positive reaction in greater dilution before it is heated. Boas has further shown that during progress of mercurial treatment a positive result can be demonstrated for a longer period by the use of unheated serum. Nevertheless, evidence has accumulated that fresh serum of non-syphilitics is apt to give a positive reaction.

It seems to me that the original method is safer for the reason that it permits of the standardization of the reagents and the putting up of adequate controls. The reaction is a quantitative one, and we consider a serum to give a positive result which gives in the presence of extract a very much more marked fixation of complement than a normal serum. We may arrive at our result either as Dr. McKenzie has suggested by varying the dose of complement or by the previous titration of the extract with a view to the determination of the dose which fixes complement with syphilitic but not with normal serum. The variations in the complement content of the guinea-pig serum are, I think, quite adequately met by the routine titration of the haemolytic system before the commencement of the actual test, and the use of a dose of haemolysin which, acting in conjunction with a particular complement-containing serum, contains three minimal lytic doses. The variations in the complement are, I think, in practice adequately compensated by varying the dose of the haemolytic amboceptor.

Reliable results can only be obtained by employing a method which allows of a standardization of the reagents employed, and in which controls can be used which disclose all possible sources of error; and although various modifications have been employed with considerable success, the evidence at our disposal is enormously in favour of the original method.

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RECOGNITION OF B. TYPHOSUS BY COMPLEMENT FIXATION.

Being a Paper read in the Section of Bacteriology at the Annual Meeting of the British Medical Association, London, July, 1910.

By H. R. Dean, M.B., B.Ch.,
Lister Institute.

The object of these experiments has been to ascertain if it is practicable to recognize by the complement fixation method the presence of small quantities of typhoid bacilli in the mixed growth obtained on plates inoculated with an emulsion of faeces.

The cases examined have been taken from among the series of typhoid carriers, or suspected carriers, examined by the usual bacteriological methods at the Lister Institute. The results obtained by me have been controlled by the results obtained by the isolation and identification of the bacteria on the customary sugar media, and I have great pleasure in recording my thanks to Dr. Ledingham for controlling my results by the isolation of typhoid in pure culture, as well as for the great interest he has taken in these experiments. The independent control thus afforded to my observations has been of the utmost service to me. The method was as follows:

Three Petri capsules of MacConkey's medium (neutral red bile salt, lactose agar) were inoculated with an emulsion of the faeces. These were the actual plates used in the bacteriological examination of the faeces, and were not subjected to any special treatment. After twenty-four hours' growth the first plate, which was in the majority of cases thickly covered with a mixed growth, was treated as follows: The entire growth on the plate was rapidly emulsified in 3 c.cm. of distilled water The emulsion was removed with a Pasteur pipette to a test tube, which was placed in the incubator at 37° C. After a period varying from three to twenty-four hours the emulsion was removed from the incubator and frozen hard in an ice and salt freezing mixture. When thoroughly frozen it was returned to the incubator at 37° C. for a further period of time, varying from one to twenty-four hours. The emulsion was then centrifugalized until clear or nearly clear. The clear supernatant fluid was pipetted off and tested for the presence of typhoid antigen with a typhoid immune serum obtained from a horse.

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[1] From the Bacteriological Department of the Lister Institute.

The details of the method are as follows: Each tube contained two and a half cubic centimetres, or five quantities each of half a cubic centimetre.

- 0.5 c.cm. containing extract diluted with physiological saline solution.
- 0 5 c.cm. containing serum diluted with physiological saline solution.
- 0.5 c.cm of a 1 in 10 dilution of fresh guinea-pig serum.
- 0 5 c.cm. of a 1 in 20 suspension of washed sheep's corpuscles.
- 0.5 c.cm. of a dilution of haemolytic serum (rabbit v. sheep corpuscles).

The haemolytic system was prepared and standardized in the usual way, and from two to three times the minimal haemolytic dose was used. The extract was used in three amounts of 0.3, 0 15, and 0.075 of a cubic centimetre diluted in each case to 0.5 c.cm. The typhoid serum was used in a dose of 0.1 c.cm. diluted to 0.5 c.cm., and the same dose of normal horse serum diluted in a similar fashion was used as a control, with each dose of extract. Further details are best seen from the following example.

Table I.

	With Typhoid Serum 0.1.	With Normal Serum 0.1.
	Haemolysis.	Haemolysis.
Extract A:		
0.3	None	Almost complete.
0.15	,,	Complete.
0.075	,,	,,
Extract B:		
0 3	Almost complete	Almost complete.
0.15	Complete	Complete.
0.075	,,	,,

Controls typhoid serum 0 2 and normal serum 0 2, without bacterial extract. The haemolysis was in both cases complete.

The extract dilutions were first made, the bulk being in each case made up to 0 5 c.cm. with normal saline solution. The extract dilutions were made in duplicate, and to one series was added 0.1 of typhoid serum diluted to ½ c.cm. To the other series was added 0.1 of normal horse serum diluted to ½ c.cm. Control tubes containing double the dose of serum (0 2) diluted to 1 c.cm. were also put up. To every tube was added 0.5 of a 1 in 10 dilution of fresh guinea-pig serum. The tubes were then incubated for one hour at 37° C. At the expiration of one hour there was added to each tube 0.5 of a 1 in 20 suspension of sheep's corpuscles and 0.5 c.cm. of a 1 in 500 dilution of haemolysin. The tubes were then incubated for one hour, and the results read and recorded.

Eighty-five plates were examined by this method, and in 81 cases the result was in agreement with the bacteriological findings. In 66 cases the complement fixation method gave a negative result, and no typhoid colonies could be isolated from the plates. In 15 cases a positive result was obtained, and typhoid bacilli were isolated in culture from the plates. In 4 cases out of 85 the result did not agree with the results obtained by the usual method. In all cases the reaction was very sharp and clearly marked, and no difficulty was experienced in reading the results.

It does not seem necessary to go into any detail as to the method, for no departure was made from the accepted technique of complement fixation except in the matter of the preparation of the bacterial extracts. These extracts, which will be again mentioned, were found to be very uniform, to have a powerful specific action, and to have little tendency to independent absorption of complement; they were, moreover, very easy to make. The optimum time for the making of an extract by this method has not been determined, but the extracts were found to be quite good which had occupied four hours in the making, and extracts which had been allowed a longer time were not appreciably more powerful. In any case, it is quite possible to make a good extract in four hours and to form an opinion as to the presence of typhoid bacilli on the same day as that on which the plates are removed from the incubator.

The results obtained seem to show that the method is accurate, and it is certainly delicate. I have no evidence as yet that it may succeed in cases where typhoid bacilli are too few for detection by ordinary methods, but in several cases from which typhoid bacilli were isolated by cultural methods with considerable difficulty a marked reaction was obtained by the complement-fixation method. The result was, in fact, quite as definite as in cases in which the typhoid colonies on the plate were numerous and obvious. I hope shortly to get more evidence on this point.

As a test of the delicacy of the reaction three agar plates were generously inoculated with *B. coli.* They were then incubated for forty-eight hours, at the end of which time one normal loop of typhoid bacilli was mixed with the growth of one plate, to another was added half a loop, and the third was untouched. Extracts were prepared by emulsifying the growth of each plate with 3 c.cm. of distilled water. The result of the complement-fixation experiment is shown in the following table:

TABLE II.

	Typhoid Serum 0.1.	Normal Serum 0.1.
	Haemolysis.	Haemolysis.
Extract A:		
0.2	None	Complete.
0.1	,,	,,
Extract B:		
0.2	,,	,,
0.1	Slight	,,
Extract C:		
0.2 ...	Almost complete	,,
0.1	Complete	,,

Controls of serum alone:
Typhoid serum 0 2 diluted to 1 c.cm. ... Complete haemolysis.
Normal serum 0.2 diluted to 1 c.cm. ... Complete haemolysis.

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[1] From the Bacteriological Department of the Lister Institute.

Each tube contained the dose of extract diluted with saline to 0.5 c.cm. *plus* the dose of serum diluted with saline to 0.5 c cm., and to each tube was added 0.5 c.cm. of a 1 in 10 dilution of fresh guinea pig serum. The tubes were incubated for one hour, and then received 0.5 c.cm. of a suspension of sheep's corpuscles, 1 in 20, and 0.5 c.cm. of haemolysin, 1 in 600. The plates A and B, which had received one and half a loop of typhoid bacilli respectively, were very plainly differentiated from C, to which typhoid had not been added.

The result shows that a small quantity of typhoid bacilli can be detected even when mixed with an enormous quantity of *B. coli.* It will be seen from the table that 0.1 c cm of extract B, which contained approximately one sixtieth of a loop of typhoid bacilli, gave a perfectly definite reaction. The recognition of typhoid bacilli in mixed culture by this method is easy and rapid, for a result can be obtained within eight hours of the removal of the incubated plate from the incubator. Moreover, it can be easily combined with the usual cultural methods.

For making the extract I have used the first of the three plates inoculated with the emulsion of faeces. The first plate was, as a rule, overgrown with confluent colonies, and quite useless for bacteriological purposes. In cases in which the colonies were discrete on the first plate, some of the suspicious colonies were picked off before the extract was made.

In 2 cases out of 85 a complement fixation reaction was obtained, but typhoid bacilli were not isolated in pure culture. In these two cases it was not possible to determine whether the serum reaction or the cultural methods had been at fault Of the other cases no difficulty was at any time experienced in deciding between a positive and a negative reaction. No cases were met with in which a positive reaction was simulated by any organism resembling typhoid. At the same time it is recognized that the number of cases investigated is far two small to exclude this possibility. It is more probable that no organisms of the typhoid group occurred in this comparatively small series of experiments. To settle this point I prepared extracts from plates made with pure cultures of paratyphoid A and B, and from a culture of aertryck. These gave a reaction which was in no way distinguishable from that obtained with a typhoid extract. It seemed to me, however, probable that this was a group reaction, comparable to that seen in agglutination experiments, and that the source of error might be eliminated by accurate dosage of the serum. To test this view it seemed advisable to experiment with extracts prepared from pure cultures of various members of the group.

It is not intended in this paper, which is of the nature of a preliminary communication, to go at all fully into the literature of the subject. It may, however, be mentioned that the specificity of the complement fixation reaction in the case of the typhoid group is at the present time generally acknowledged as the result of the work of numerous authors. The question of specificity as regards the typhoid and the paratyphoid bacilli was settled by the papers of Leuchs and the very interesting results to be obtained by the absorption of the group ambocepter were first recorded by Simnitzky.

The strains of bacilli used in these experiments were tested by the usual sugar tests.

The method of preparing the extract was as follows: A Roux bottle, with an agar surface of about 20 × 10 cm., was freely inoculated and incubated for twenty-four hours. At the end of this time the growth was emulsified in 20 c.cm. of distilled water. The emulsion was put into an incubator at 37° C. for twenty-four hours, at the end of which time it was frozen hard with a mixture of ice and salt. It was allowed to thaw after two hours, and then returned to the incubator for another twenty four hours, when it was again frozen and thawed. The emulsion was then centrifugalized and a clear or almost clear fluid obtained. The clear fluid was poured off and kept in the cold room, and was found to remain fit for use for at least one month. In those cases in which the extract was not quite clear after centrifugalization it was found that a deposit settled to the bottom of the flask after a few days, leaving the supernatant fluid quite clear.

The extracts made in this way gave good results with the complement fixation method, and were very free from any tendency to absorb complement spontaneously. It will be noticed that the extract was not heated above 37° C. at any period during its manufacture; nevertheless, cultures made from the clear fluid immediately after centrifugalization were either sterile or showed only one or two colonies. The extracts were almost invariably found to be sterile after they had been a few days in the cold room.

In this experiment it will be noted that with a dosage of 0.1 c.cm. typhoid serum it is impossible to differentiate between typhoid extract and extracts prepared from para typhoid A and B. When, however, the dose of serum is reduced to 0 025, the positive reaction with the paratyphoid extracts almost disappears, while the reaction with typhoid extract is reduced only to a very small extent. A very similar result is shown in Table IV.

These experiments show that in complement fixation, as in agglutination, there is a group reaction which, when the antiserum is used in large amount, tends to mask the specific reaction between typhoid antibody and typhoid antigen. The complement fixation by a typhoid extract and typhoid antiserum is an extremely delicate and specific reaction, to demonstrate which it is, however, necessary to adjust carefully the proportions of the ingredients. In this case a dose of 0 025 c.cm. gives, under the conditions mentioned, a reaction with typhoid extract which is little short of the maximum, while the reaction with the other members of the group almost disappears.

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[1] From the Bacteriological Department of the Lister Institute.

TABLE III.

		Typhoid Serum, 0.1 c.cm.	Typhoid Serum, 0.025 c.cm.	Normal Serum, 0.1 c.cm.
		Haemolysis.	Haemolysis.	Haemolysis.
	Typhoid Extract "M" c.cm.			
1	0.1	None	None	Complete
2	0.05	„	„	„
3	0.025	„	„	„
4	0.0125	„	„	„
5	0.00625	„	Trace	„
6	0.003125	Trace	Almost complete	„
7	0.0015625	Marked	Complete	„
8	0.00078125	Almost complete	„	„
9	0.000390625	Complete	„	„
	Paratyphoid A Extract			
1	0.1	None	Trace	omplete
2	0.05	„	Almost complete	„
3	0.025	„	Complete	„
4	0.0125	„	„	„
5	0.00625	„	„	„
6	0.003125	Trace	„	„
7	0.0015625	Almost complete	„	„
8	0.00078125	Complete	„	„
9	0.000390625	„	„	„
	Paratyphoid B Extract "Sch."			
1	0.1	None	None	Complete
2	0.05	„	Almost complete	„
3	0.025	„	Complete	„
4	0.0125	„	„	„
5	0.00625	Trace	„	„
6	0.003125	Slight	„	„
7	0.0015625	Almost complete	„	„
8	0.00078125	Complete	„	„
9	0.000390625	„	„	„

Controls:

Typhoid serum (without extract) 0.2	...	Complete haemolysis.
Normal serum (without extract) 0.2	...	Complete haemolysis.

To each tube was added 0.5 of a 1 in 10 dilution of fresh guinea-pig serum, and, after incubation for one hour, 0.5 c.cm. of a 1 in 20 suspension of washed sheep's corpuscles and 0.5 c.cm. of a 1 in 600 dilution of haemolysin. The extracts used in these experiments were prepared at the same time and by the same method from approximately equal amounts of fresh cultures.

TABLE IV.

		Typhoid Serum, 0.1 c.cm.	Typhoid Serum, 0.25 c.cm.	Normal Serum, 0.1 c.cm.
		Haemolysis.	Haemolysis.	Haemolysis.
	Typhoid Extract "T." c.cm.			
1	0.1	None	None	Almost complete
2	0.05	„	„	Complete
3	0 025	„	„	„
4	0.0125	„	„	„
5	0.00625	„	Slight	„
6	0.003125	Trace	Almost complete	„
7	0.0015625	Slight	Complete	„
8	0.00078125	Complete	„	„
9	0.000390625	„	„	„
	Paratyphoid B Extract "Mac."			
1	0.1	None	Marked	Almost complete
2	0.05	„	Complete	Complete
3	0.025	„	„	„
4	0.0125	„	„	„
5	0.00625	„	„	„
6	0.003125	„	„	„
7	0 0015625	Slight	„	„
8	0.00078125	Almost complete	„	„
9	0.000390625	Complete	„	„
	Aertryck Extract.			
1	0.1	None	Slight	Almost complete
2	0.05	„	Marked	Complete
3	0.025	Trace	Complete	„
4	0.0125	Slight	„	„
5	0.00625	Marked	„	„
6	0.003125	Almost complete	„	„
7	0.0015625	Complete	„	„
8	0.00078125	„	„	„
9	0.000390625	„	„	„

The controls of typhoid serum 0.2 c.cm., and normal serum 0.2 c.cm., without extract, in either case showed complete haemolysis.

To each tube was added 0.5 c.cm. of a 1 in 10 dilution of fresh guinea-pig serum, and after one hour's incubation, 0.5 c.cm. of a 1 in 20 suspension of sheep's corpuscles and 0.5 c.cm. of haemolysin 1 in 300.

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[1] From the Bacteriological Department of the Lister Institute.

TABLE V.

		Typhoid Serum Untreated 0.1 c.cm	Typhoid Serum Previously Absorbed with Paratyphoid B 0 1 c.cm	Typhoid Serum Previously Absorbed with Typhoid 0.1 c.cm.	Normal Serum.
		Haemolysis.	Haemolysis.	Haemolysis.	Haemolysis.
	Typhoid Extract M. c cm.				
1	0 1	None	None	Almost complete	Complete.
2	0 05	,,	,,	Complete	,,
3	0.025	,,	,,	,,	,,
4	0.0125	,,	Trace	,,	,,
5	0.00625	Trace	Almost complete	,,	,,
6	0.003125	Almost complete	Complete	,,	,,
7	0.0015625	Complete	,,	,,	,,
8	0.00078125	,,	,,	,,	,,
9	0 000390625	,,	,,	,,	,,
	Paratyphoid A Extract.				
1	0.1	None	Almost complete	Almost complete	Complete.
2	0.05	,,	Complete	Complete	,,
3	0 025	,,	,,	,,	,,
4	0.0125	Trace	,,	,,	,,
5	0.00625	Almost complete	,,	,,	,,
6	0.003125	Complete	,	,,	,,
7	0.0015625	,,	,,	,,	,,
8	0.00078125	,,	,,	,,	,,
9	0.000390625	,,	,,	,,	,,
	Paratyphoid B Extract.				
1	0.1	None	Almost complete	Almost complete	Complete.
2	0.05	,,	Complete	Complete	,,
3	0.025	,,	,,	,,	,,
4	0.0125	Trace	,,	,,	,,
5	0 00625	Almost complete	,,	,,	,,
6	0.003125	Complete	,,	,,	,,
7	0.0015625	,,	,,	,,	,,
8	0.00078125	,,	,,	,,	,,
9	0 000390625	,,	,,	,,	,,
	Serum alone.				
	0.2	Complete	Almos complete	Almost complete	Complete,
	0.1	,,	Complete	Complete	,,

To each tube was added 0 5 c.cm. of a 1 in 10 dilution of fresh guinea-pig serum. The experiment was incubated for an hour, and there were subsequently added 0.5 c.cm of a 1 in 20 suspension of sheep's corpuscles and 0.5 c.cm. of a 1 in 400 dilution of haemolysin.

In the following experiment (Table V), an attempt was made still further to demonstrate the specific nature of the reaction.

Two Roux bottles, which had been inoculated with a culture of typhoid, and two which had been inoculated with a culture of paratyphoid B, were incubated for twenty-four hours. The growth was then washed off from each pair of bottles with about 200 c.cm. of saline solution. The emulsions were then centrifugalized, and deposits of washed bacilli were obtained. To each deposit was added 20 c.cm. of a 1 in 5 dilution of typhoid serum, and the thoroughly emulsified mixtures—serum and bacilli—were left for one hour in the cold room. The mixtures were then rapidly centrifugalized, and the almost clear serum mixtures were at once decanted. There were thus obtained an antityphoid serum which had been acted on by typhoid bacilli for one hour in the cold, and an antityphoid serum which had been similarly treated with paratyphoid B bacilli. These serums were then compared with a specimen of the same antityphoid serum, similarly diluted but otherwise untreated.

From this experiment it appears that absorption with paratyphoid B removes the group antibody from an antityphoid serum, and that a serum treated in this way gives no complement fixation with extracts of either paratyphoid A or B, while the reaction with typhoid extract is only slightly reduced. It will be noticed that previous treatment of antityphoid serum with paratyphoid B extract removes not only the antibodies for paratyphoid B, but also the antibodies for paratyphoid A.

On the other hand, previous treatment with typhoid bacilli removes the amboceptor for typhoid as well as for paratyphoid A and B. I hope to be able to continue these experiments, and more especially in the direction of the recognition of typhoid bacilli in mixed culture.

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[1] From the Bacteriological Department of the Lister Institute.

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15

Studies in Complement Fixation with Strains of Typhoid, Paratyphoid, and Allied Organisms.

By H. R. DEAN.[1]

THE practical applications of the complement-fixation method may be divided under two headings: (1) The recognition of antibody with the aid of a known antigen; (2) the identification of an unknown antigen with the aid of known antibody. Under the second heading fall the very numerous researches which have been undertaken with a view to the finer differentiation of closely allied bacteria. If value is to be set on this differentiation method of classification it must be shown that it is absolutely specific.

Although the majority of workers have come to the conclusion that the complement-fixation method is extremely specific, a considerable number of papers have appeared in which it is claimed that differentiation may be impossible between races of bacteria usually recognized as distinct. Although a few workers have gone so far as to say that the method is useless for purposes of differentiation, the majority have only found difficulty in distinguishing between nearly allied organisms. In such cases reference is very frequently made to the presence of a group reaction which renders a sharp distinction difficult of attainment.

For a full account of the literature of the subject the reader is referred to the article on "Complement Fixation," by Meier (1909), in Weichardt's "Jahresbericht."

The differentiation of *Bacillus typhosus* from *Bacillus coli* was recorded in Bordet and Gengou's (1902) original paper. These authors immunized guinea-pigs with cultures of *Bacillus typhosus* and tested the serum so obtained with emulsions of *Bacillus coli* and emulsions of *Bacillus typhosus*. They found that complement was completely absorbed by relatively small amounts of typhoid bacillary emulsion in the presence of antityphoid serum, but that relatively large amounts of

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[1] From the Bacteriological Department of the Lister Institute.

Bacillus coli emulsion mixed with the same antityphoid serum were necessary to produce even a partial fixation of complement. They concluded that the sensitizing substances of antityphoid serum were markedly but not absolutely specific. These results were confirmed in the same year by Widal and Le Sourd (1902). The differentiation of *Bacillus typhosus* and *Bacillus coli* by this method is also described by Ballner and Reibmayr (1908), Simnitzky (1908), and H. R. Dean (1910).

The introduction by Wassermann (1905) of bacillary extracts in place of the previously used emulsions was followed by numerous papers dealing with complement fixation. Further improvements in the method of preparation of bacterial extracts were devised by Leuchs (1907), who was able to satisfactorily differentiate *Bacillus typhosus* from *Bacillus paratyphosus*, although his results showed considerable evidence of a strong group reaction. The results of Leuchs, as regards *Bacillus typhosus* and *Bacillus paratyphosus*, were confirmed by Ballner and Reibmayr (1908), who also found that differentiation was rendered difficult by the presence of a marked group reaction. They advised the use of greatly diluted antisera. They were able to distinguish *Bacillus typhosus* from *Bacillus coli*, and either of these organisms from *Bacillus paratyphosus* B or from *Bacillus typhi murium;* they were, however, unable to distinguish between *Bacillus paratyphosus* B and *Bacillus typhi murium.* An *antityphi murium* serum gave a marked reaction with extract of *Bacillus typhi murium* and an almost equally marked reaction with extract of *Bacillus paratyphosus* B. An antityphoid or an anticoli serum was found to give a strong reaction with its homologous extract and a less, but still evident "group reaction" with extracts of *Bacillus typhi murium* and *Bacillus paratyphosus* B. The *Bacillus paratyphosus* B and the *Bacillus typhi murium* extracts were found to give an identical "group reaction" with a heterologous serum. The group reactions observed by the agglutination and complement-fixation methods ran a parallel course. They considered that the complement-fixation method was as sharp and as specific as agglutination, but that it was not to be preferred as it was so much more complicated, and that consequently sources of error were numerous.

Simnitzky (1908) saturated an antityphoid serum with *Bacillus coli* and with *Bacillus paratyphosus* without removing the antibodies specific for *Bacillus typhosus.* If, however, the serum was treated with typhoid bacilli the complement binding properties for *Bacillus typhosus* were removed together with the group antibodies for *Bacillus paratyphosus* and *Bacillus coli*.

Posner (1908) worked with the serum of patients suffering from typhoid and paratyphoid infections. He concluded that the complement-fixation method was unsuitable for the differentiation of typhoid and paratyphoid infections.

Spät (1908), who also made use of clinical material, came to the conclusion that complement fixation did not afford a means of differentiating between the infections caused by members of the typhoid and paratyphoid group.

From these papers it appears that considerable difficulty has been experienced in the differentiation of typhoid from paratyphoid. Specific differences are recorded by Leuchs and Ballner and Reibmayr, who, however, found it necessary to employ very dilute antisera. The presence of a group reaction is described in both these papers and to this group reaction may probably be attributed the negative results published by Spät and Posner.

The experiments published by H. R. Dean (1910) showed that with the low dilutions of antiserum no differentiation was possible between *Bacillus typhosus, Bacillus paratyphosus* B and *Bacillus paratyphosus* A. When, however, the antiserum was highly diluted complete differentiation was obtained. Experiments were also published which confirmed the absorption experiments of Simnitzky.

One of the most useful applications of the complement-fixation method has been the attempt to classify the various strains which have been isolated from cases of food-poisoning. Sacquépeé (1907) was able to effect a separation into two groups which he called "Enteritis I" and "Enteritis II." The group Enteritis I comprises *Bacillus Gaertner, Bacillus Morseele* and *Bacillus Bruxelles.* The group Enteritis II comprises *Bacillus Aertryck, Bacillus Posen, Bacillus Düsseldorf, Bacillus Sirault,* the bacillus of hog-cholera, *Bacillus psittacosis,* and *Bacillus paratyphosus* B.

A serum prepared by immunization with any organism in Group I gave a marked reaction with any other member of Group I, but no reaction with any member of Group II. In the same way a serum prepared by immunization with any organism of Group II was specific in its action and reacted equally well with any of the members of Group II, but not with the members of Group I.

Pitt (1909) investigated the *Bacillus nodulifaciens bovis* (Langer) and decided that it should be included in the Gaertner group. He was, however, unable to distinguish *Bacillus Gaertner* from *Bacillus typhosus* by the complement-fixation method, and for this reason concluded that it was questionable if complement fixation was more delicate than the previously employed serological tests.

The classification of the organisms forming the food-poisoning group has recently been very fully investigated by Altmann (1910). This author, as a result of agglutination experiments, divided the Salmonella group into two sub-groups: Enteritis I—Paratyphoid B (Schottmüller), the bacillus of hog-cholera, *Bacillus psittacosis*, *Bacillus typhi murium*, the so-called "Schweinepest" bacillus, *Bacillus pseudo-tuberculosis* of guinea-pigs, and food-poisoning bacilli of the type of Flügge, that is to say, *Bacillus Aertryck*. Enteritis II—The rat virus organisms of the type of Danysz, Dunbar, and Issatschenko, food-poisoning bacilli of the type of *Bacillus Morseele*. For the purpose of his complement-fixation experiments Altmann employed seventeen strains from Group I, namely, eight strains of *Bacillus paratyphosus* B (Schottmüller), two strains of so-called "Schweinepest" bacilli, one strain of *Bacillus typhi murium*, one strain of *Bacillus pseudo-tuberculosis*, and one strain of *Bacillus psittacosis*. Five strains of Group II were used, namely, the strains *Morseele* and *Bruges*, and the three rat poisons, *Dunbar*, *Ratin*, and *Danysz*. Extracts of these organisms were made by Leuchs' method. Using a paratyphoid B serum, the author obtained a marked reaction with each of the seventeen strains from Group I, no reaction with the strains of Group II, and no reaction with *Bacillus typhosus* and *Bacillus coli*. An exactly similar result was obtained by the use of a serum derived from an animal immunized with *Bacillus suipestifer*.

Although the author states that no differentiation was obtained between the various strains of Group I, the published table shows that very considerable differences in the intensity of the reaction occurred. By the use of a serum derived from an animal immunized with *Bacillus Morseele*, Altmann obtained a marked reaction with the extracts prepared from the members of Group II, but no reaction with the extracts prepared from the strains included under Group I. He concludes that further differentiation within the two groups is impossible.

Bainbridge (1911) has shown that by the use of Castellani's absorption method it is possible to distinguish *Bacillus paratyphosus* B (Schottmüller) from *Bacillus Aertryck*. *Bacillus paratyphosus* B is associated by Bainbridge with paratyphoid fever, while the typical symptoms of meat-poisoning are associated with a bacillus which is in every respect identical with *Bacillus Aertryck*. No differentiation, however, is possible between *Bacillus Aertryck*, *Bacillus suipestifer*, and the bacillus associated with the epizootic disease of guinea-pigs.

It will be seen that Bainbridge's results are opposed to those of Altmann, who was unable to differentiate between *Bacillus paratyphosus* B and *Bacillus Aertryck.*

From these and other papers on the subject it appears that the complement-fixation method is a means of demonstrating a highly specific reaction between a bacillary extract and its homologous antiserum. The specific reaction is, however, in considerable danger of being masked by the presence of a more or less strong group reaction. The group reaction, while it adds considerably to the difficulties of differentiation, is not without interest, from the insight which it affords as to the degree of relationship between nearly allied organisms.

GENERAL OBJECT OF THE EXPERIMENTS.

The object of the experiments which are to be described has been:—

(1) To determine the conditions under which the most complete differentiation can be affected.

(2) To determine the influence of relative excess of antigen and of relative excess of antibody on the absorption of complement.

(3) To determine if it is possible, by the use of the complement-fixation method, to differentiate *Bacillus paratyphosus* B (Schottmüller) from *Bacillus Aertryck.*

METHODS.

Preparation of Bacterial Extracts.—All the extracts used in these experiments were prepared by the same method. Roux bottles containing nutrient agar were freely inoculated from a broth culture and kept in the incubator at 37° C. for forty-eight hours. The agar surface of such bottles is about 20 cm. by 10 cm. After forty-eight hours, the growth in each bottle was rapidly emulsified with 20 c.c. of distilled water. The bacterial emulsion was then decanted into a sterile flask and subsequently pipetted off into sterile test-tubes, in amounts of about 5 c.c. to each tube. In some cases the emulsion was treated for one hour at 60° C. This preliminary heating was not found to have any effect favourable or unfavourable on the properties of the extract, but it is recommended, as it obviates risk of infection. The tubes containing the bacterial emulsion were, after a thorough shaking, immersed in an ice and salt freezing mixture. The emulsion was allowed to freeze hard, and then to thaw slowly at room temperature. The emulsion was usually prepared in the morning, and after the first freezing was allowed to stand at room temperature, with occasional shaking, until the evening.

when the freezing process was repeated. After the emulsion had been thawed for the second time, it was placed in a strong bottle containing glass beads and shaken all night in a shaking-machine. On the second day the emulsion was pipetted in quantities of 5 c.c. into test-tubes, and the freezing and thawing process was repeated in the morning and in the evening. During the second night the emulsion was again thoroughly shaken in a strong bottle containing glass beads. On the third day the emulsion was centrifugalized. The supernatant fluid, which was either quite clear or only very slightly opalescent, was decanted into a sterile flask and stored for use in the cold room.

Antisera.—The antisera were prepared by the intravenous injection of rabbits with saline emulsions of twenty-four-hour agar cultures. Each rabbit received four or five injections at intervals of four or five days, and was tested on the eighth day after the last injection. If it was found to be satisfactory, the animal was bled out on the ninth or tenth day. The serum, which had separated after twenty-four hours, was heated for half an hour at 56° C. The inactivated serum was stored in small sealed glass bottles, each of which contained about 2 c.c.

THE HÆMOLYTIC SYSTEM.

The hæmolytic system consisted of washed sheep corpuscles, hæmolytic serum derived from rabbits which had been injected with sheep corpuscles, and fresh guinea-pig serum. The guinea-pig serum was freshly prepared on the same day as the experiments were carried out. The hæmolytic system was titrated on each occasion, and an amount of hæmolysin was used in the complement-fixation experiment, which contained about four minimal lytic doses.

EXPERIMENTAL METHODS.

The bulk of fluid in every tube was 2·5 c.c., made up of five separate quantities, each of 0·5 c.c. Each tube contained 0·5 c.c. of diluted bacterial extract (antigen), 0·5 c.c. of diluted serum (antibody), and 0·5 c.c. of a 1 in 10 dilution of fresh guinea-pig serum (complement). After one hour's incubation at 37° C., there were added to each tube 0·5 c.c. of the previously determined dilution of hæmolytic serum, and 0·5 c.c. of a 1 in 20 suspension of washed sheep corpuscles. The tubes were then incubated for a period varying between forty-five minutes and two hours. The series of tubes was removed from the incubator, when the controls showed complete hæmolysis.

The strains used in this series of experiments were as follows:

Three strains of *Bacillus typhosus* isolated by Dr. Ledingham from typhoid carriers, and designated Math., T., and Mie. Four strains of *Bacillus paratyphosus* B, designated Schott., MacW., Ma, and P.9. One strain of *Bacillus paratyphosus* A, one strain of *Bacillus enteritidis*, Gaertner, three strains from cases of food-poisoning in men, designated Düsseldorf, H.A., and A.3, one strain isolated from food and one strain isolated from a healthy pig.

All the strains except the typhoid and the Gaertner strains were given to me by Dr. Bainbridge. The strains were carefully tested before the commencement and at frequent intervals during the course of the experiments. In all cases the typical sugar reactions were obtained.

ANALYSIS OF RESULTS.

The results of the experiments are recorded in the annexed tables :—

Table I: In the experiment illustrated in Table I an antityphoid serum has been titrated with an extract made from another strain of *Bacillus typhosus*. It will be seen that this serum acts best in a dilution of 1 in 20 or 1 in 40, that in the dilutions of 1 in 80 and in 1 in 160 the reaction is less but still very marked. A very fair result is obtained in a dilution of 1 in 320, while in a dilution of 1 in 640 the fixation of complement is hardly appreciable. The effect of excess of antibody may be seen by comparing the results in the first three rows. It will be seen that a dilution of 1 in 20 is more effective than a dilution of 1 in 10 and that a dilution of 1 in 10 is more effective than a dilution of 1 in 5. This effect is evidenced in the tubes 5, 6 and 7 of the first three rows. In tube 7, row A, hæmolysis was almost complete; in tube 7, row C, there was only the slightest trace of hæmolysis. This result indicates that when the extract is diluted 1 in 320 and the serum 1 in 5, an excess of antibody is present which prevents the fixation of complement. In tube 7 of row C the same amount of antigen is present, but only one-quarter of the amount of antibody. In this tube the complement was almost completely fixed. The effect of excess of antigen is more difficult to demonstrate because it is a difficult matter to prepare extracts rich in antigen and because large doses of bacterial extract have a great tendency to spontaneous absorption of complement. A slight indication of this effect can, however, be made out in row G, tubes 1 and 2. In these tubes the amounts of extract present were 0·1 c.c. and 0·05 c.c. respectively. These amounts appear to represent a slight antigen excess, for a trace of hæmolysis was noticed. When the amount of extract was reduced (tubes 4, 5 and 6) no hæmolysis occurred. The amount of serum

was the same in all tubes of row G. From this experiment it is evident that a quantitative relationship exists between antigen and antibody and that excess of either constituent is unfavourable to the fixation of complement. In working with bacterial extracts the inhibitory influence of excess of antigen is of theoretical rather than of practical interest, for

TABLE I.—TITRATION OF ANTI-

Serum prepared by injecting a rabbit intravenously with *Bacillus typhosus*

	Dilutions of typhoid extract "Math." 0·5 c.c.	DILUTIONS OF ANTITYPHOID			
		A 0·5 c.c. of 1 in 5	B 0·5 c.c. of 1 in 10	C 0·5 c.c. of 1 in 20	D 0·5 c.c. of 1 in 40
1	1 in 5	No hæmolysis	No hæmolysis	No hæmolysis	No hæmolysis
2	1 in 10	,, ,,	,, ,,	,, ,,	,, ,,
3	1 in 20	,, ,,	,, ,,	,, ,,	,, ,,
4	1 in 40	,, ,,	,, ,,	,, ,,	,, ,,
5	1 in 80	Trace hæmolysis	,, ,,	,, ,,	,, ,,
6	1 in 160	Marked hæmolysis	Trace hæmolysis	,, ,,	,, ,,
7	1 in 320	Almost complete hæmolysis	Marked hæmolysis	Trace hæmolysis	Trace hæmolysis
8	1 in 640	Complete hæmolysis	Almost complete hæmolysis	Marked hæmolysis	Marked hæmolysis
9	1 in 1,280	,, ,,	Complete hæmolysis	Almost complete hæmolysis	Complete hæmolysis
10	1 in 2,560	,, ,,	,, ,,	Complete hæmolysis	,, ,,
11	0·5 c.c. normal saline serum control	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis

Nine parallel rows, each consisting of eleven tubes, were set up. In the tubes numbered 1 to 10, in all nine rows, were put successive dilutions of extract. The bulk of extract dilution was 0·5 c.c. in each tube. The tubes numbered 1 in all nine rows contained the same amount of extract, namely, 0·1 c.c. of extract diluted to 0·5 c.c. with normal saline. The tubes numbered 2 in all rows contained half as much extract diluted to the same bulk, that is to say, 0·05 extract diluted with normal saline to 0·5 c.c. and so on. Half a cubic centimetre of normal saline was then added to all tubes in the ninth row, and to the tubes it is a difficult matter to make an extract sufficiently rich in antigen. The possibility of a relative excess of antiserum is a matter of more practical importance. For instance, if the object of the experiment be to detect a very small quantity of antigen, the optimal dilution of the antiserum must be determined by a preliminary test of the antiserum

SERUM WITH HOMOLOGOUS EXTRACT.

(Strain T.). The typhoid extract was prepared from Strain M.

HEATED RABBIT SERUM (STRAIN T.)				I 0·5 c.c. normal saline (extract control)
E 0·5 c.c. of 1 in 80	F 0·5 c.c. of 1 in 160	G 0·5 c.c. of 1 in 320	H 0·5 c.c. of 1 in 640	
No hæmolysis	No hæmolysis	Trace hæmolysis	Slight hæmolysis	Complete hæmolysis
,, ,,	,, ,,	,, ,,	Marked hæmolysis	,, ,,
,, ,,	,, ,,	No hæmolysis	,, ,,	,, ,,
,, ,,	,, ,,	,, ,,	Almost complete hæmolysis	,, ,,
,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
,, ,,	Slight hæmolysis	Half hæmolysed	Complete hæmolysis	,, ,,
Slight hæmolysis	Marked hæmolysis	Marked hæmolysis	,, ,,	,, ,,
Marked hæmolysis	Almost complete hæmolysis	Complete hæmolysis	,, ,,	,, ,,
Complete hæmolysis	Complete hæmolysis	,, ,,	,, ,,	,, ,,
,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
Complete hæmolysis	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis	

numbered 11 in all the other rows. The tubes in the ninth row (I) constituted the extract controls. The tubes numbered 11 constituted serum controls. The tubes in rows A to H received each half a cubic centimetre of the serum dilution mentioned at the top of each column. To every tube was added 0·5 c.c. of a 1 in 10 dilution of fresh guinea-pig serum. The experiment was then incubated for one hour, and then 0·5 c.c. of a 1 in 20 suspension of sheep corpuscles and 0·5 of a 1 in 600 dilution of hæmolysin were added.

with the homologous extract. Unless this is done the antiserum will not be given the best chance of detecting a trace of antigen. Such a preliminary titration also serves the more obvious purpose of ascertaining how far the particular serum may be diluted. For the differentiation of two nearly allied organisms, the highest dilution should be chosen which gives a well-marked reaction with the homologous extract. A knowledge of the antibody content of a serum is best obtained by titrating the serum with the homologous extract in such a way that each dilution of serum is allowed to interact with each dilution of extract. It is always advisable to carry out a preliminary test of this nature with every serum, as the

TABLE II.—SERUM ANTITYPHOID (STRAIN T.). EXTRACTS OF TYPHOID (STRAIN MATH.

	Extract dilutions 0·5 c.c. of	TYPHOID EXTRACT (STRAIN MATH.)			TYPHOID EXTRACT (STRAIN "T.")			BACILLUS AERTRYCK EXTRACT (STRAIN AE)		
		+ 0·5 c.c. antityphoid serum 1 in 10	+ 0·5 c.c. antityphoid serum 1 in 100	+ 0·5 c.c. normal saline	+ 0·5 c.c. antityphoid serum 1 in 10	+ 0·5 c.c. antityphoid serum 1 in 100	+ 0·5 c.c. normal saline	+ 0 5 c.c. antityphoid serum 1 in 10	+ 0·5 c.c. antityphoid serum 1 in 100	+ 0·5 c.c. normal saline solution
1	1 in 5	No hæmolysis	No hæmolysis	Complete hæmolysis	No hæmolysis	No hæmolysis	Complete hæmolysis	No hæmolysis	Complete hæmolysis	Complete hæmolysis
2	1 in 10	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
3	1 in 20	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
4	1 in 40	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
5	1 in 80	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
6	1 in 160	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
7	1 in 320	Trace hæmolysis	,, ,,	,, ,,	Trace hæmolysis	,, ,,	,, ,,	Trace hæmolysis	,, ,,	,, ,,
8	1 in 640	Marked hæmolysis	Trace hæmolysis	,, ,,	Complete hæmolysis	Trace hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,
9	1 in 1280	Complete hæmolysis	Marked hæmolysis	,, ,,	,, ,,	Almost complete hæmolysis	,, ,,	Almost complete hæmolysis	,, ,,	,, ,,

0·1 c.c., 0·05 c.c., 0·01 c.c. and 0·005 c.c. of typhoid antiserum were put up without extract as serum controls; complete hæmolysis took place in every tube.

From each extract were made three parallel sets of dilutions. To the first row was added antityphoid serum 1 in 10, to the second row antityphoid serum 1 in 100, to the third row knowledge so gained is of use in determining the dose of antiserum appropriate for use in subsequent experiments.

Table II: This table illustrates the reaction between an antityphoid serum and extracts prepared from strains of *Bacillus typhosus*, *Bacillus paratyphosus*, and a strain of *Bacillus Aertryck*. With each extract three sets of dilutions were made. To one set no serum was added, and this set served the purpose of an extract control. The second set received antityphoid serum in a dilution of 1 in 100, while the third set received antityphoid serum in a dilution of 1 in 10. An examination of the table shows that with a serum dilution of 1 in 10 no differentiation

AND T.), OF PARATYPHOID A AND OF PARATYPHOID B (STRAINS SCHOTT. AND MACW.).

EXTRACT OF PARATYPHOID A			EXTRACT OF PARATYPHOID B (STRAIN SCHOTT.)			EXTRACT OF PARATYPHOID B (STRAIN MACW.)		
+ 0·5 c.c. antityphoid serum 1 in 10	+ 0·5 c.c. antityphoid serum 1 in 100	+ 0·5 c.c. normal saline	+ 0·5 c.c. antityphoid serum 1 in 10	+ 0·5 c.c. antityphoid serum 1 in 100	+ 0·5 c.c. normal saline	+ 0·5 c.c. antityphoid serum 1 in 10	+ 0·5 c.c. antityphoid serum 1 in 100	+ 0·5 c.c. normal saline
No hæmolysis	Almost complete hæmolysis	Almost complete hæmolysis	No hæmolysis	Complete hæmolysis	Complete hæmolysis	No hæmolysis	Almost complete hæmolysis	Almost complete hæmolysis
,, ,,	,, ,,	Complete hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,	Complete hæmolysis	Complete hæmolysis
,, ,,	Complete hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
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,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
Trace hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	Trace hæmolysis	,, ,,	,, ,,
Almost complete hæmolysis	,, ,,	,, ,,	Almost complete hæmolysis	,, ,,	,, ,,	Half hæmolysed	,, ,,	,, ,,
Almost complete hæmolysis	,, ,,	,, ,,	Complete hæmolysis	,, ,,	,, ,,	Complete hæmolysis	,, ,,	,, ,,

normal saline solution. The third row constituted the extract control. To every tube was added 0·5 c.c. of a 1 in 10 dilution of fresh guinea-pig serum. The experiment was then incubated for one hour, and then there were added 0·5 c.c. of a 1 in 20 suspension of washed sheep corpuscles and a 1 in 500 dilution of hæmolytic serum.

is possible between *Bacillus typhosus*, *Bacillus paratyphosus* A, *Bacillus paratyphosus* B, and *Bacillus Aertryck.* When, however, a 1 in 100 dilution of the serum is employed the typhoid extract is completely differentiated from the other extracts.

Table III: This table illustrates the titration of a serum derived from a rabbit, which had been injected with paratyphoid A bacilli. A comparison of row A with row B shows the influence of excess of antibody in diminishing the reaction.

TABLE III.—ANTISERUM DERIVED FROM RABBIT AFTER INTRAVENOUS INJECTION OF PARATYPHOID A.

Extract prepared from the same strain of Paratyphoid A.

	Paratyphoid A extract, 0·5 c.c. of a dilution	A + 0·5 c.c. of paratyphoid A antiserum dilution 1 in 5	B + 0·5 c.c. of paratyphoid A antiserum dilution 1 in 20	C + 0·5 c.c. of 1 in 80 dilution of antiserum	D + 0·5 c.c. of 1 in 320 dilution of antiserum	E + 0·5 c.c. of 1 in 5 dilution of normal serum	F + 0·5 c.c. normal saline
1	1 in 20	No hæmolysis	No hæmolysis	No hæmolysis	No hæmolysis	Complete hæmolysis	Complete hæmolysis
2	1 in 40	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
3	1 in 80	Trace hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
4	1 in 160	Complete hæmolysis	Trace hæmolysis	Trace hæmolysis	Almost complete hæmolysis	,, ,,	,, ,,
5	1 in 320	,, ,,	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis	,, ,,	,, ,,

As a further control, 2 in 5, 1 in 5, and 1 in 10 dilutions of extract were prepared. No antiserum was added to these tubes, but the bulk was made up with 0·5 c.c. normal saline solution. Dilution 2 in 5 showed marked hæmolysis, dilutions 1 in 5 and 1 in 10 showed complete hæmolysis. The serum controls of tubes containing single and double the amounts of serum used in the corresponding columns of the experiment, but without the addition of bacillary extract. All the serum controls showed complete hæmolysis.

In this experiment six identical and parallel rows were prepared so that the tubes of the same number in each row contained the same dilution of extract. For instance, tube 1 in every row contained 0·025 extract diluted to 0·5 c.c. with saline solution. Every tube of row A then received 0·5 c.c. of a 1 in 5 dilution of antiserum. To every tube of row B was added 0·5 c.c. of a 1 in 20 dilution, to every tube of row C 0·5 c.c. of a 1 in 80 dilution, and to every tube of row D 0·5 c.c. of a 1 in 320 dilution of antiserum. Each of the tubes of row E received 0·5 c.c. of a 1 in 5 dilution of normal serum, and in row F each tube received 0·5 c.c. of normal saline solution. All tubes then received 0·5 c.c. of a 1 in 10 dilution of fresh guinea-pig serum, and the experiment was then incubated for one hour. At the expiration of the hour there were added 0·5 c.c. of a 1 in 20 suspension of washed sheep corpuscles and 0·5 c.c. of a 1 in 1,000 dilution of hæmolytic serum.

Table IV: This table shows the group reaction obtained by the use of a large amount of serum (dilution 1 in 20) and the complete differentiation obtained by the use of a small amount of serum (dilution 1 in 200).

Table V: This table illustrates the titration of a serum obtained from a rabbit, which had been injected with *Bacillus paratyphosus* B, with the homologous extract.

Table VI: Extracts prepared from *Bacillus typhosus*, *Bacillus paratyphosus* A, *Bacillus paratyphosus* B, and *Bacillus Aertryck* are titrated with paratyphoid B serum in four separate dilutions. With a dilution of 1 in 10 no differentiation is possible; with a dilution of 1 in 50 a great difference is perceptible between the extracts of *Bacillus paratyphosus* B and *Bacillus Aertryck* as compared with the extracts of *Bacillus typhosus* and *Bacillus paratyphosus* A. When the serum is diluted 1 in 100 the reaction with *Bacillus typhosus* and *Bacillus paratyphosus* A almost disappears, but, with this dilution of serum *Bacillus paratyphosus* B cannot be differentiated from *Bacillus Aertryck*. With a dilution of 1 in 200, however, *Bacillus paratyphosus* B is satisfactorily differentiated from *Bacillus Aertryck*, as well as from *Bacillus typhosus* and *Bacillus paratyphosus* B.

Table VII: It is here shown that by the use of a 1 in 300 dilution of the paratyphoid A serum two strains of *Bacillus paratyphosus* B are differentiated from strains of *Bacillus typhosus*, *Bacillus paratyphosus* A, *Bacillus Aertryck*, and *Bacillus enteritidis*, Gaertner.

Table VIII: The extracts employed in this experiment were prepared from strains of the Paratyphoid-Aertryck group, isolated from various sources: Düsseldorf—a strain isolated from a case of food-poisoning in man. B.A.—isolated from food. Heyd. Ali.—a strain isolated from man (nature of illness unknown). M.A.—strain isolated from healthy man. P.R. 9—strain isolated from paratyphoid carrier. 840—Strain isolated from healthy pig. The sera used in this experiment (Table VIII) were the paratyphoid B serum used in the experiments of Tables V, VI, and VII, and a serum (designated O'B.) obtained from a rabbit injected with a strain derived from a guinea-pig. This strain, which was isolated from a case of epizootic disease among guinea-pigs was identical in sugar reactions and agglutination tests with *Bacillus Aertryck*. For all these strains and for the serum O'B. I am indebted to the kindness of Dr. Bainbridge. Table VIII shows that the strains M.A. and P.R. 9 are to be regarded as paratyphoid B. The other four strains, namely, Düsseldorf, B.A., Heyd. Ali., and 840, react

TABLE IV.

The serum used in this experiment was obtained from a rabbit after injection with paratyphoid A. The extracts were prepared from the same strain of paratyphoid A, from two strains of typhoid (Math. and T.), and from two strains of paratyphoid B (Schott. and MacW.).

	0·5 c.c. of extract dilutions	Typhoid extract (strain Math.) + 0·5 c.c. normal saline		Typhoid extract (strain T.) + 0·5 c.c. normal saline		Paratyphoid A extract + 0·5 c.c. normal saline		Paratyphoid B extract (strain Schott.) + 0·5 c.c. normal saline		Paratyphoid B extract (strain MacW.) + 0·5 c.c. normal saline	
1	2 in 5	Marked hæmolysis ...		Half hæmolysed ...		Half hæmolysed... ...		Marked hæmolysis ...		Half hæmolysed	
2	1 in 5	Complete hæmolysis ...		Complete hæmolysis ...		Almost complete hæmolysis		Complete hæmolysis ...		Complete hæmolysis	
3	1 in 10	,, ,, ...		,, ,, ...		Complete hæmolysis ...		,, ,, ...		,, ,,	
		Paratyphoid A serum		Paratyphoid A serum		Paratyphoid A serum		Paratyphoid A serum		Paratyphoid A serum	
		+ 0·5 c.c. of dilution 1 in 20	+ 0·5 c.c. of dilution 1 in 200	+ 0·5 c.c. of dilution 1 in 20	+ 0·5 c.c. of dilution 1 in 200	+ 0·5 c.c. of dilution 1 in 20	+ 0·5 c.c. of dilution 1 in 200	+ 0·5 c.c. of dilution 1 in 20	+ 0·5 c.c. of dilution 1 in 200	+ 0·5 c.c. of dilution 1 in 20	+ 0·5 c.c. of dilution 1 in 200
4	1 in 5	No hæmolysis	Complete hæmolysis	No hæmolysis	Complete hæmolysis	No hæmolysis	Trace hæmolysis	No hæmolysis	Complete hæmolysis	No hæmolysis	Marked hæmolysis
5	1 in 10	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	No hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,
6	1 in 20	,, ,,	,, ,,	Trace hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
7	1 in 40	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	Trace hæmolysis	,, ,,	,, ,,	,, ,,
8	1 in 80	Marked hæmolysis	,, ,,	Almost complete hæmolysis	,, ,,	Half hæmolysed	Trace hæmolysis	,, ,,	,, ,,	Trace hæmolysis	Almost complete hæmolysis
9	1 in 160	Almost complete hæmolysis	,, ,,	Complete hæmolysis	,, ,,	Marked hæmolysis	Half hæmolysed	Complete hæmolysis	,, ,,	Almost complete hæmolysis	Complete hæmolysis
10	1 in 320	Complete hæmolysis	,, ,,	,, ,,	,, ,,	Complete hæmolysis	Almost complete hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,

Serum control tubes containing 0·5, 0·025 and 0·005 c.c. of serum, without extract, showed complete hæmolysis. Tubes 1, 2 and 3 in each row were extract controls. The series of dilutions 4 to 10 were made in duplicate for each extract. To each tube of the first row was added 0·5 c.c. of a 1 in 20 dilution of the serum of a rabbit which had been immunized with paratyphoid A. To each tube of the second row was added 0·5 c.c. of a 1 in 200 dilution of the same serum. To every tube was added 0·5 c.c. of a 1 in 10 dilution of fresh guinea-pig serum. After one hour's incubation every tube received 0·5 c.c. of a 1 in 20 suspension of sheep blood corpuscles and 0·5 c.c. of 1 in 500 dilution of hæmolytic serum.

TABLE V.—TITRATION OF SERUM FROM RABBIT INJECTED WITH PARATYPHOID B (SCHOTT.) WITH THE HOMOLOGOUS EXTRACT.

	0·5 c.c. of dilutions of paratyphoid B extract	A + 0·5 c.c. of a 1 in 5 dilution of paratyphoid B serum	B + 0·5 c.c. of a 1 in 100 dilution of paratyphoid B serum	C + 0·5 c.c. of a 1 in 200 dilution of paratyphoid B serum	D + 0·5 c.c. of a 1 in 400 dilution of paratyphoid B serum	E + 0·5 c.c. of a 1 in 800 dilution of paratyphoid B serum	F + 0·5 c.c. of a 1 in 1600 dilution of paratyphoid B serum	G + 0·5 c.c. of a 1 in 5 dilution of normal rabbit serum	H + 0·5 c.c. of normal saline solution
1	2 in 5	No hæmolysis	No hæmolysis	No hæmolysis	No hæmolysis	No hæmolysis	No hæmolysis	Half hæmolysed	Half hæmolysed
2	1 in 5	,, ,,	,, ,,	,, ,,	,, ,,	Trace hæmolysis	Trace hæmolysis	,, ,,	Complete hæmolysis
3	1 in 10	,, ,,	,, ,,	,, ,,	,, ,,	No hæmolysis	,, ,,	Marked hæmolysis	—
4	1 in 20	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	No hæmolysis	Almost complete hæmolysis	—
5	1 in 40	,, ,,	,, ,,	,, ,,	,, ,,	Trace hæmolysis	Trace hæmolysis	Complete hæmolysis	—
6	1 in 80	,, ,,	,, ,,	,, ,,	Trace hæmolysis	Half hæmolysed	Almost complete hæmolysis	,, ,,	—
7	1 in 160	Trace hæmolysis	Trace hæmolysis	Trace hæmolysis	Half hæmolysed	Almost complete hæmolysis	Complete hæmolysis	,, ,,	—
8	1 in 320	Complete hæmolysis	Half hæmolysed	Half hæmolysed	Almost complete hæmolysis	Complete hæmolysis	,, ,,	,, ,,	—
9	1 in 640	,, ,,	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis	,, ,,	—	,, ,,	—

Serum controls, 0·2 c.c. and 0·1 c.c., of paratyphoid B serum showed complete hæmolysis. While 0·1 c.c. of normal serum showed complete hæmolysis, a little complement was absorbed in the control tube containing 0·2 c.c. normal serum. This tendency of the normal serum to absorb complement accounts for the readings of tubes 2 to 4 in row G.

In this experiment eight identical series of dilutions were made. To each row was added a different dilution of the homologous serum. To every tube was added 0·5 c.c. of a 1 in 10 dilution of fresh guinea-pig serum. After the completion of one hour in the incubator, 0·5 c.c. of a 1 in 20 suspension of sheep corpuscles and 0·5 c.c. of a 1 in 600 dilution of hæmolysin were added to every tube.

TABLE VI.

	0·5 c.c. of extract dilutions	Typhoid extract (strain Math.) + 0·5 c.c. of a 1 in 10 dilution of paratyphoid B serum	Paratyphoid A extract + 0·5 c.c. of a 1 in 10 dilution of paratyphoid B serum	Paratyphoid B extract (strain Schott.) + 0·5 c.c. of a 1 in 10 dilution of paratyphoid B serum	Aertryck extract + 0·5 c.c. of a 1 in 10 dilution of paratyphoid B serum
1	1 in 10	No hæmolysis	No hæmolysis	No hæmolysis	No hæmolysis
2	1 in 20	,, ,,	,, ,,	,, ,,	,, ,,
3	1 in 40	,, ,,	,, ,,	,, ,,	,, ,,
4	1 in 80	,, ,,	,, ,,	,, ,,	,, ,,
5	1 in 160	Trace hæmolysis	,, ,,	Trace hæmolysis	Trace hæmolysis
6	1 in 320	Almost complete hæmolysis	Trace hæmolysis	Half hæmolysed	Half hæmolysed
7	1 in 640	Complete hæmolysis	Marked hæmolysis	Almost complete hæmolysis	Complete hæmolysis
8	1 in 1280	,, ,,	Complete hæmolysis	Complete hæmolysis	,, ,,
		Typhoid extract + 0·5 c.c. of a 1 in 50 dilution of paratyphoid B serum	Paratyphoid A extract + 0·5 c.c. of a 1 in 50 dilution of paratyphoid B serum	Paratyphoid B extract + 0·5 c.c. of a 1 in 50 dilution of paratyphoid B serum	Aertryck extract + 0·5 c.c. of a 1 in 50 dilution of paratyphoid B serum
1	1 in 10	No hæmolysis	No hæmolysis	No hæmolysis	No hæmolysis
2	1 in 20	Trace hæmolysis	,, ,,	,, ,,	,, ,,
3	1 in 40	Half hæmolysed	Trace hæmolysis	,, ,,	,, ,,
4	1 in 80	Marked hæmolysis	Almost complete hæmolysis	,, ,,	,, ,,
5	1 in 160	Complete hæmolysis	,, ,,	,, ,,	,, ,,
6	1 in 320	,, ,,	Complete hæmolysis	Trace hæmolysis	Trace hæmolysis
7	1 in 640	,, ,,	,, ,,	,, ,,	Almost complete hæmolysis
8	1 in 1280	,, ,,	,, ,,	Complete hæmolysis	Complete hæmolysis

TABLE VI—(*continued*).

	0·5 c.c. of extract dilutions	Typhoid extract + dilution of 1 in 100 paratyphoid B serum	Paratyphoid A extract + 0·5 c.c. of a 1 in 100 paratyphoid B serum	Paratyphoid B extract + 1 in 100 paratyphoid B serum	Aertryck extract + 0·5 c.c. of a 1 in 100 dilution of paratyphoid B serum
1	1 in 10	No hæmolysis	No hæmolysis	No hæmolysis	No hæmolysis
2	1 in 20	Almost complete hæmolysis	Half hæmolysed	,, ,,	,, ,,
3	1 in 40	Complete hæmolysis	Almost complete hæmolysis	,, ,,	,, ,,
4	1 in 80	,, ,,	Complete hæmolysis	,, ,,	,, ,,
5	1 in 160	,, ,,	,, ,,	,, ,,	Trace hæmolysis
6	1 in 320	,, ,,	,, ,,	Trace hæmolysis	Almost complete hæmolysis
7	1 in 640	,, ,,	,, ,,	Half hæmolysed	Complete hæmolysis
8	1 in 1280	,, ,,	,, ,,	Complete hæmolysis	,, ,,
		Typhoid extract + 0·5 c.c. of a 1 in 200 dilution of paratyphoid B serum	**Paratyphoid A extract + 0·5 c.c. of a 1 in 200 dilution of paratyphoid B serum**	**Paratyphoid B extract + 0·5 c.c. of a 1 in 200 dilution of paratyphoid B serum**	**Aertryck extract + 0·5 c.c. of a 1 in 200 dilution of paratyphoid B serum**
1	1 in 10	Marked hæmolysis	Almost complete hæmolysis	No hæmolysis	Trace hæmolysis
2	1 in 20	Complete hæmolysis	Complete hæmolysis	,, ,,	Marked hæmolysis
3	1 in 40	,, ,,	,, ,,	,, ,,	Complete hæmolysis
4	1 in 80	,, ,,	,, ,,	,, ,,	,, ,,
5	1 in 160	,, ,,	,, ,,	Trace hæmolysis	,, ,,
6	1 in 320	,, ,,	,, ,,	Almost complete hæmolysis	,, ,,
7	1 in 640	,, ,,	,, ,,	,, ,,	,, ,,
8	1 in 1280	,, ,,	,, ,,	Complete hæmolysis	,, ,,

Extract controls, without serum, in dilutions of 1 in 5, 1 in 10, and 1 in 20, and serum controls in dilutions of 1 in 5, 1 in 10 and 1 in 20 were put up. All six control tubes showed complete hæmolysis.

With each extract four identical series of dilutions were made. From the paratyphoid B antiserum four dilutions were made—1 in 10, 1 in 50, 1 in 100, 1 in 200. All four extracts were tested in a series of dilutions with each of the four serum dilutions. 0·5 c.c. of fresh guinea-pig serum 1 in 10 was added to each tube. After one hour's incubation each tube received 0·5 c.c. of a 1 in 20 suspension of sheep corpuscles and 0·5 c.c. of a 1 in 500 dilution of hæmolysin.

with the O'B. serum, but not with the paratyphoid B serum. From a consideration of Tables VI, VII, and VIII it appears that the complement-fixation method allows of a differentiation between *Bacillus paratyphosus* B and *Bacillus Aertryck.* This conclusion is in agreement with that which has been reached by Bainbridge (1911) who employed the absorption method of Castellani.

TABLE VII.

To all tubes 0·5 c.c. of a 1 in 300 dilution of paratyphoid B serum obtained from a rabbit immunized with paratyphoid B (strain Schott.).

	0·5 c.c. extract dilutions	Paratyphoid A extract	Paratyphoid B extract (strain Schott.)	Paratyphoid B extract (strain MacW.)	Aertryck extract (strain AE)	Gaertner extract	Typhoid extract (strain Math.)
1	1 in 20	Complete hæmolysis	No hæmolysis	No hæmolysis	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis
2	1 in 40	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
3	1 in 80	,, ,,	Slight hæmolysis	Slight hæmolysis	,, ,,	,, ,,	,, ,,
4	1 in 160	,, ,,	Complete hæmolysis	Complete hæmolysis	,, ,,	,, ,,	,, ,,

Extract dilutions of 1 in 5, 1 in 10, and 1 in 20 were put up as extract controls for each of the six extracts employed. Serum controls, dilutions 1 in 100 and 1 in 300, were also put up. Complete hæmolysis was obtained in all the control tubes.

In this experiment each extract was put up in four dilutions. To every tube, except the controls, was added 0·5 c.c. of a 1 in 300 dilution of paratyphoid B serum. To every tube was added 0·5 c.c. of a 1 in 10 dilution of fresh guinea-pig serum. After one hour's incubation each tube received 0·5 c.c. of a 1 in 20 suspension of sheep's corpuscles and 0·5 c.c. of a 1 in 1,000 dilution of hæmolysin.

GENERAL DISCUSSION OF RESULTS.

The Tables I to VIII shows very clearly that the complement-fixation method affords an extremely delicate and specific means of separating and identifying closely allied races of bacteria. Absolute differentiation can, however, only be secured by the careful determination of the dosage of antigen and antibody. The dilution of antiserum necessary for a differentiation experiment can be ascertained by the titration of the antiserum with the homologous extract. As a rule satisfactory differentiation can be obtained with the greatest dilution of antiserum

TABLE VIII.

	0·5 c.c. extract dilutions	Extract of strain "Düsseldorf"	Extract of strain B.A.	Extract of strain Heyd. Ali.	Extract of strain M.A.	Extract of strain 840	Extract of strain P.R.9
		+ 0·5 c.c. of dilution 1 in 50 of serum (strain O'B.)					
1	1 in 5	No hæmolysis	Trace hæmolysis	Trace hæmolysis	Almost complete hæmolysis	No hæmolysis	Complete hæmolysis
2	1 in 10	Trace hæmolysis	,, ,,	,, ,,	Complete hæmolysis	,, ,,	,, ,,
3	1 in 20	Half hæmolysed	Half hæmolysis	Almost complete hæmolysis	,, ,,	Trace hæmolysis	,, ,,
4	1 in 40	Almost complete hæmolysis	Almost complete hæmolysis	,, ,,	,, ,,	Almost complete hæmolysis	,, ,,
		+ 0·5 c.c. of a 1 in 200 dilution of paratyphoid B serum					
1	1 in 5	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis	No hæmolysis	Complete hæmolysis	Trace hæmolysis
2	1 in 10	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
3	1 in 20	,, ,,	,, ,,	,, ,,	Half hæmolysed	,, ,,	Almost complete hæmolysis
4	1 in 40	,, ,,	,, ,,	,, ,,	Complete hæmolysis	,, ,,	Complete hæmolysis
		+ 0·5 c.c. normal saline solution (extract control)					
1	1 in 5	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis	Half hæmolysed	Complete hæmolysis	Complete hæmolysis
2	1 in 10	,, ,,	,, ,,	,, ,,	Complete hæmolysis	,, ,,	,, ,,
3	1 in 20	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
4	1 in 40	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,

In this experiment six extracts made from six separate strains were used. Each extract was put up in four dilutions. Each extract was tested with each of the two sera. Strains M.A. and P.R.9 reacted with the paratyphoid serum, but not with the serum prepared with strain O'B. The other four strains reacted with the O'B. serum. Serum control tubes containing single and double doses of serum without extract showed complete hæmolysis. To every tube was added 0·5 c.c. of a 1 in 10 dilution of fresh guinea-pig serum, and after one hour in the incubator 0·5 c.c. of a 1 in 20 suspension of sheep corpuscles and 0·5 c.c. of a 1 in 500 dilution of hæmolysin.

which is found to give a thoroughly satisfactory reaction with the homologous extract.

With low dilutions of antiserum a marked group reaction is obtained. An examination of Table VI will show that the intensity of the group reaction corresponds with the closeness of the relationship between the strains employed. In this experiment the paratyphoid A and the typhoid extracts were easily differentiated from the paratyphoid B extract. It was only when the serum was still further diluted that *Bacillus Aertryck* could be differentiated from *Bacillus paratyphosus* B.

Table IX.

	0·5 c.c. of extract dilutions	Untreated antiparatyphoid (B) serum 1 in 20	Antiparatyphoid B serum 1 in 20 absorbed with typhoid emulsion	Antiparatyphoid B serum 1 in 20 absorbed with an emulsion of paratyphoid A	Antiparatyphoid B serum 1 in 20 absorbed with an emulsion of paratyphoid B	Normal saline
	Typhoid extract					
1	1 in 10	No hæmolysis	Complete hæmolysis	Almost complete hæmolysis	Almost complete hæmolysis	Complete hæmolysis
2	1 in 20	,, ,,	,, ,,	Complete hæmolysis	Complete hæmolysis	,, ,,
3	1 in 40	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
4	1 in 80	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
5	1 in 160	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
6	1 in 320	Trace hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,
7	1 in 640	Almost complete hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,
8	1 in 1280	Complete hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,
	Paratyphoid A extract					
1	1 in 10	No hæmolysis	Almost complete hæmolysis	Marked hæmolysis	Almost complete hæmolysis	Complete hæmolysis
2	1 in 20	,, ,,	Complete hæmolysis	Almost complete hæmolysis	,, ,,	,, ,,
3	1 in 40	,, ,,	,, ,,	Complete hæmolysis	,, ,,	,, ,,
4	1 in 80	,, ,,	,, ,,	,, ,,	Complete hæmolysis	,, ,,
5	1 in 160	Trace hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,
6	1 in 320	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
7	1 in 640	Complete hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,
8	1 in 1280	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,

Table IX—(*continued*).

	0·5 c.c. of extract dilutions	Untreated antiparatyphoid (B) serum 1 in 20	Antiparatyphoid B serum 1 in 20 absorbed with typhoid emulsion	Antiparatyphoid B serum 1 in 20 absorbed with an emulsion of paratyphoid A	Antiparatyphoid B serum 1 in 20 absorbed with an emulsion of paratyphoid B	Normal saline
	Paratyphoid B extract					
1	1 in 10	No hæmolysis	No hæmolysis	No hæmolysis	Almost complete hæmolysis	Complete hæmolysis
2	1 in 20	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
3	1 in 40	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
4	1 in 80	,, ,,	,, ,,	,, ,,	Complete hæmolysis	,, ,,
5	1 in 160	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
6	1 in 320	Marked hæmolysis	Trace hæmolysis	Half hæmolysed	,, ,,	,, ,,
7	1 in 640	Complete hæmolysis	Half hæmolysed	Complete hæmolysis	,, ,,	,, ,,
8	1 in 1280	,, ,,	Complete hæmolysis	,, ,,	,, ,,	,, ,,
Serum controls (without extract)						
Single dose ...		0·5 c.c. Complete hæmolysis	0·5 c.c. Complete hæmolysis	0·5 c.c. Complete hæmolysis	0·5 c.c. Complete hæmolysis	—
Double dose...		1 c.c. Complete hæmolysis	1 c.c. Complete hæmolysis	1 c.c. Almost complete hæmolysis	1 c.c. Complete hæmolysis	—

Notes on Table IX.—To each tube in column 1 was added ½ c.c. of a 1 in 20 dilution of an antiparatyphoid B serum. The absorbed serum used in the next column was prepared as follows: Two Roux bottles were inoculated with *Bacillus typhosus* and incubated twenty-four hours. The growth was emulsified in saline and washed and centrifugalized three times. To the washed deposit were added 10 c.c. of saline (previously cooled to 4° C.), and then 10 c.c. of a 1 in 10 dilution of the serum (previously cooled to 4° C.). The mixture was thoroughly shaken and left for half an hour in the cold room. It was then centrifugalized until clear and pipetted off. The serums used in column 3 and column 4 were prepared in the same fashion with emulsions of paratyphoid A and paratyphoid B bacilli.

In the actual experiment each tube contained ½ c.c. of extract dilution, ½ c.c. of serum dilution and ½ c.c. of a 1 in 10 dilution of fresh guinea-pig serum. After incubation for one hour there were added ½ c.c. of hæmolytic serum diluted 1 in 500 and ½ c.c. of a 1 in 20 suspension of sheep corpuscles.

The degree of relationship between the various strains was also evidenced by the time in which hæmolysis occurred in the tubes containing serum and heterologous extract. For example, in the experiment illustrated in Table VII hæmolysis occurred first in the rows of tubes containing the Gaertner and the typhoid extract. The row containing paratyphoid A extract was next hæmolysed, and it was only after a considerable interval that hæmolysis occurred in the row of tubes containing the Aertryck extract. In the experiment figured in Table VI a similar phenomenon was observed; in the tubes containing the serum dilution 1 in 200 the order of onset of hæmolysis was (1) typhoid extract, (2) paratyphoid A extract, (3) Aertryck extract, at a considerably longer interval. The same phenomenon was observed in the experiments figured in Tables II and IV.

When an animal is immunized with a particular strain of organism it may be presumed that a large number of chemical substances capable of acting as antigens are introduced. The majority of these are specific and peculiar to the organism in question; others, it may be assumed, are common to the organism and to other members of the group. The group reaction may be attributed to the presence in the antiserum of a number of antibodies produced by the injection of these group antigens.

COMPLEMENT FIXATION AFTER PRELIMINARY ABSORPTION OF THE GROUP ANTIBODIES.

In Tables IX and X there are illustrated two experiments in which the group antigens were removed by saturating an antiserum with a thick emulsion of a bacillus closely allied to the homologous strain. It will be seen from Table IX that, when a paratyphoid B serum is absorbed with an emulsion of paratyphoid A bacilli, the serum loses its capacity for binding complement in the presence of either a paratyphoid A extract or a typhoid extract. That is to say, the group antibodies are removed. In the same way, absorption with an emulsion of typhoid bacilli removes the group reaction both for typhoid and for paratyphoid A. After absorption with a paratyphoid B emulsion the group antibodies are found to be removed, together with the antibodies specific for paratyphoid B. Table X illustrates a similar experiment with an antityphoid serum.[1]

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[1] It is important that the bacilli used for absorbing the "group" antibody should be thoroughly washed and that the absorption process should be carried out at a low temperature. Unless these precautions are taken it will be found that the serum possesses the property of "spontaneously" fixing complement. This phenomenon is presumably due to the fact that antigen is dissolved out of the bodies of the bacilli and unites with the antibodies of the serum. The antigen antibody complex thus formed renders the serum quite unfitted for subsequent use.

TABLE X.

		+ 0·5 c.c. of 1 in 5 dilution of typhoid serum untreated	+ 0·5 c.c. of 1 in 5 dilution of typhoid serum previously absorbed with paratyphoid B	+ 0·5 c.c. of 1 in 5 dilution of typhoid serum previously absorbed with typhoid	+ 0·5 c.c. of 1 in 5 dilution of normal serum
	Dilutions of typhoid extract M				
1	1 in 5	No hæmolysis	No hæmolysis	Almost complete hæmolysis	Complete hæmolysis
2	1 in 10	,, ,,	,, ,,	Complete hæmolysis	,, ,,
3	1 in 20	,, ,,	,, ,,	,, ,,	,, ,,
4	1 in 40	,, ,,	Trace hæmolysis	,, ,,	,, ,,
5	1 in 80	Trace hæmolysis	Almost complete hæmolysis	,, ,,	,, ,,
6	1 in 160	Almost complete hæmolysis	Complete hæmolysis	,, ,,	,, ,,
7	1 in 320	Complete hæmolysis	,, ,,	,, ,,	,, ,,
8	1 in 640	,, ,,	,, ,,	,, ,,	,, ,,
9	1 in 1280	,, ,,	,, ,,	,, ,,	,, ,,
	Paratyphoid A extract				
1	1 in 5	No hæmolysis	Almost complete hæmolysis	Almost complete hæmolysis	Complete hæmolysis
2	1 in 10	,, ,,	Complete hæmolysis	Complete hæmolysis	,, ,,
3	1 in 20	,, ,,	,, ,,	,, ,,	,, ,,
4	1 in 40	Trace hæmolysis	,, ,,	,, ,,	,, ,,
5	1 in 80	Almost complete hæmolysis	,, ,,	,, ,,	,, ,,
6	1 in 160	Complete hæmolysis	,, ,,	,, ,,	,, ,,
7	1 in 320	,, ,,	,, ,,	,, ,,	,, ,,
8	1 in 640	,, ,,	,, ,,	,, ,,	,, ,,
9	1 in 1280	,, ,,	,, ,,	,, ,,	,, ,,
	Paratyphoid B extract				
1	1 in 5	No hæmolysis	Almost complete hæmolysis	Almost complete hæmolysis	Complete hæmolysis
2	1 in 10	,, ,,	Complete hæmolysis	Complete hæmolysis	,, ,,
3	1 in 20	,, ,,	,, ,,	,, ,,	,, ,,
4	1 in 40	Trace hæmolysis	,, ,,	,, ,,	,, ,,
5	1 in 80	Almost complete hæmolysis	,, ,,	,, ,,	,, ,,
6	1 in 160	Complete hæmolysis	,, ,,	,, ,,	,, ,,
7	1 in 320	,, ,,	,, ,,	,, ,,	,, ,,
8	1 in 640	,, ,,	,, ,,	,, ,,	,, ,,
9	1 in 1280	,, ,,	,, ,,	,, ,,	,, ,,
	Serum alone				
	2 in 5	Complete hæmolysis	Almost complete hæmolysis	Almost complete hæmolysis	Complete hæmolysis
	1 in 5	,, ,,	Complete hæmolysis	Complete hæmolysis	,, ,,

To each tube was added 0·5 c.c. of a 1 in 10 dilution of fresh guinea-pig serum. The experiment was incubated for an hour, and there were subsequently added 0·5 c.c. of a 1 in 20 suspension of sheep corpuscles and 0·5 c.c. of a 1 in 400 dilution of hæmolysin.

Table XI.—Titration of Antityphoid Serum (Strain T.) with Typhoid Extract (Strain Math.).

	0·5 c.c. of dilution of typhoid extract (strain Math.)	0·5 c.c. of dilutions of typhoid serum (strain T.)					F + 0·5 c.c. normal saline
		A 4 in 5	B 2 in	C 1 in 40	D 1 in 160	E 1 in 320	
1	4 in 5	No hæmolysis	No hæmolysis	Trace hæmolysis	Half hæmolysed	Half hæmolysed	Half hæmolysed
2	2 in 5	,, ,,	,, ,,	No hæmolysis	,, ,,	Almost complete hæmolysis	Complete hæmolysis
3	1 in 5	,, ,,	,,	,, ,,	Trace hæmolysis	Half hæmolysed	,, ,,
4	1 in 10	Trace hæmolysis	,, ,,	,, ,,	No hæmolysis	Trace hæmolysis	,, ,,
5	1 in 20	Slight hæmolysis	Trace hæmolysis	,, ,,	,, ,,	,, ,,	,, ,,
6	1 in 40	Almost complete hæmolysis	Slight hæmolysis	,, ,,	Trace hæmolysis	Marked hæmolysis	,, ,,
7	1 in 80	Complete hæmolysis	Half hæmolysed	Trace hæmolysis	,, ,,	Almost complete hæmolysis	,, ,,
8	1 in 160	,, ,,	Complete hæmolysis	Slight hæmolysis	Half hæmolysed	,, ,,	,, ,,
9	1 in 320	,, ,,	,, ,,	Almost complete hæmolysis	Almost complete hæmolysis	Complete hæmolysis	,, ,,
10	1 in 640		,, ,,	Complete hæmolysis	Complete hæmolysis	,, ,,	,, ,,
		Serum controls					
11	Normal saline, 0·5 c.c.	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis	—

A, B, C, D, E and F represent six parallel rows of test-tubes. The tubes bearing the same number in each row contained identical extract dilutions made up to a bulk of 0·5 c.c. To each tube of row A was then added 0·5 c.c. of a 4 in 5 dilution of antiserum. To each tube of row B was added 0·5 c.c. of a 2 in 5 dilution of antiserum, and so on. The tubes in row F contain extract only and acted as extract controls. The tubes numbered 11 in the rows A to F were serum controls. To every tube was added 0·5 c.c. of a 1 in 10 dilution of fresh guinea-pig serum. After incubation for one hour every tube received 0·5 c.c. of a 1 in 20 suspension of washed sheep corpuscles and 0·5 c.c. of a 1 in 500 of hæmolytic serum.

TABLE XII.

	Serum dilutions	Typhoid serum		Paratyphoid A serum		Paratyphoid B serum	
		A	B	A	B	A	B
1	4 in 5	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis	Complete hæmolysis
2	2 in 5	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
3	1 in 5	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,	,, ,,
4	1 in 10	,, ,,	,, ,,	,, ,,	Marked hæmolysis	,, ,,	,, ,,
5	1 in 20	,, ,,	Slight hæmolysis	,, ,,	No hæmolysis	,, ,,	Trace hæmolysis
6	1 in 40	,, ,,	No hæmolysis	,, ,,	,, ,,	,, ,,	No hæmolysis

For each serum two duplicate rows of dilutions were prepared, so that each tube contained the diluted serum in a bulk of ½ c.c. To all tubes in the rows marked A was added ½ c.c. of normal saline solution. To all tubes in rows B was added 1 c.c. of normal saline. To the tubes in rows A was added ½ c.c. of a 1 in 500 dilution of a hæmolytic serum. This represented three minimal lytic doses of the particular hæmolytic serum. All the tubes then received ½ c.c. of a 1 in 20 suspension of sheep corpuscles and ½ c.c. of a 1 in 10 dilution of fresh guinea-pig serum. The experiment was incubated for two hours, and the results were recorded as above. It will be seen by an examination of the rows marked B that the hæmolytic titre of the typhoid and of the paratyphoid B antiserum was 1 in 10, while the paratyphoid A antiserum had a hæmolytic titre of 1 in 5.

The rows marked A contained the indicated doses of the antiserum together with three lytic doses of a hæmolytic serum. Although all the tubes in all three of the A rows showed complete hæmolysis at the end of two hours, there was a marked difference in the rate of hæmolysis between the tubes in each series. In each row A tube 6 was the first to hæmolyse and tube 1 the last. The slowing of hæmolysis in the tubes containing the large amounts of antiserum was very slight, but was sufficient to allow the inference that the presence of a large amount of serum is unfavourable rather than favourable to hæmolysis.

Table XI illustrates an experiment intended to demonstrate the effect of the presence of excess of antigen and antibody. The influence of relative excess of antiserum is well shown in the first two columns A and B. The effect of excess of antigen begins to be noticed in tube 1 of row C and is more marked in the first three tubes of rows D and E. The experiment shows very clearly that if the optimal effect is to be obtained antigen and antibody must be present in certain definite proportions. The influence of relative excess of either constituent is a matter of some interest from a theoretical standpoint. The effect of antiserum excess might be attributed to the reinforcement of the hæmolytic system by the "normal" hæmolysin present in the antiserum. The hæmolytic tube of the antisera used in these experiments is given in Table XII.

It seems, however, more probable that this inhibition by excess is comparable to that seen in other serum reactions.

It will be remembered that excess of antiserum causes a very similar inhibitory effect in agglutination, precipitation and hæmolysis experiments. The phenomenon is quite analogous to that described by Neisser and Wechsberg (1901) and it might be anticipated that both results might find a common explanation. Neisser and Wechsberg suggested that the free amboceptors took up or deviated a certain portion of the complement and that not enough remained to satisfy those amboceptors which had united with bacilli.

This explanation has not been universally accepted as the cause of the interference with bacteriolysis, and it seems almost impossible to apply it to the closely parallel interference with the fixation of complement. To do so it is necessary to assume that the free amboceptors deviate complement from the bound amboceptors merely to give it up again, later on, to the combined hæmolytic amboceptor.

Further experiments are being carried out at the present time on the quantitative relations of antigen and antibody and it is proposed to return to the question in a subsequent communication.

SUMMARY AND CONCLUSIONS.

(1) The complement-fixation method affords an extremely delicate and specific means of differentiating between various members of the typhoid and paratyphoid group.

(2) Very closely allied organisms can only be differentiated after experiments sufficiently extensive to secure a knowledge of the dilutions most suitable to the serum and extracts in use.

(3) By the use of the complement-fixation method *Bacillus typhosus*, *Bacillus paratyphosus* A, and *Bacillus paratyphosus* B can be easily differentiated from one another.

(4) By the use of a suitable dilution of serum it is possible to differentiate *Bacillus paratyphosus* B from *Bacillus Aertryck*.

This series of experiments entirely confirms the classification of Bainbridge.

(5) The group reaction and the degree of relationship between members of the group can be demonstrated by the use of various dilutions of an antiserum in the presence of extracts made from homologous and heterologous strains.

(6) Under certain circumstances the degree of relationship can be seen during the course of an experiment by observing the time of onset of hæmolysis in the tubes containing the various extracts.

(7) The group antibodies can be removed from the serum by a suitably arranged absorption experiment.

(8) Excess either of antiserum or of bacterial extract has an unfavourable influence on the fixation of complement.

(9) The maximal fixation of complement is obtained when antigen and antibody are present in certain definite proportions.

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17

N° 16

LXV.—*Synthesis of* dl-3 : 4-*Dihydroxyphenylalanine.*

By Casimir Funk.

The question of the formation of adrenaline in the animal body has engaged much attention. The two amino-acids that suggest themselves as the parent substances of adrenaline are obviously tyrosine and phenylalanine, but certain considerations render it probable that the body is incapable of employing these substances for the formation of adrenaline. Thus, in cases of abnormal metabolism, tyrosine is eliminated as homogentisic acid (Wolkow and Baumann, *Zeitsch. physiol. Chem.*, 1891, **15**, 228; Falta and Langstein, *ibid.*, 1903, **37**, 513; Abderhalden, Bloch, and Rona, *ibid.*, 1907, **52**, 435), whilst no catechol derivatives have been obtained from any of the aromatic amino-acids.

In the experiments which have been conducted on this point, investigators have sought to bring about a transformation of tyrosine (Ewins and Laidlaw, *J. Physiol.*, 1910, **40**, 275) into adrenaline by incubating the former substance with suprarenal gland tissue. It may well be, however, that the formation of adrenaline takes place in several steps, and that different tissues of the body are necessary.

In order to be certain that adrenaline is really formed, it is necessary to find a chemical method by which this substance can be separated from amino-acids, and such a separation is at present being attempted.

That some substance of the nature of tyrosine or phenylalanine is the precursor of adrenaline is further suggested by the fact that all three compounds belong to the same optical (*l*) series.

The synthesis of 3:4-dihydroxyphenylalanine has been effected with the idea that it may be the parent substance of adrenaline; experiments are also being conducted in order to ascertain whether any of this substance is contained in proteins. That this may be

III. Previous plague history of the villages infected in each epidemic.

IV. Previous plague history of villages infected at the beginning of each epidemic.

- A. Rohtak District.
    - (*a*) Epidemic of 1903—04.
    - (*b*) ,, ,, 1904—05.
    - (*c*) ,, ,, 1905—06.
    - (*d*) ,, ,, 1906—07.
- B. Mozuffarnagar District.
    - (*a*) Epidemic of 1903—04.
    - (*b*) ,, ,, 1904—05.
    - (*c*) ,, ,, 1905—06.
    - (*d*) ,, ,, 1906—07.
- C. Amritsar District.
    - (*a*) Epidemic of 1902—03.
    - (*b*) ,, ,, 1903—04.
    - (*c*) ,, ,, 1904—05.
    - (*d*) ,, ,, 1905—06.
    - (*e*) ,, ,, 1906—07.
- D. Summary.

V. Later plague history of the villages infected in the course of the milder epidemics.

- A. Rohtak District.
    - (*a*) Epidemic of 1903—04.
    - (*b*) ,, ,, 1905—06.
- B. Mozuffarnagar District.
    - (*a*) Epidemic of 1902—03.
    - (*b*) ,, ,, 1905—06.
- C. Summary.

VI. Later plague history of villages infected at the end of each epidemic.

- A. Rohtak District.
    - (*a*) End of epidemic of 1903—04.
    - (*b*) ,, ,, 1904—05.
    - (*c*) ,, ,, 1905—06.
- B. Mozuffarnagar District.
    - (*a*) End of epidemic of 1902—03.
    - (*b*) ,, ,, 1903—04.
    - (*c*) ,, ,, 1904—05.
    - (*d*) ,, ,, 1905—06.
- C. Amritsar District.
- D. Summary.

VII. The question whether plague tends to recur in villages in successive epidemics.

I. Introduction.

A. *Scope of observations.*

It is well established that plague, once it has gained a foothold in a place, tends to recur every year at the same season and that the plague season varies in different places. It has been demonstrated that in Bombay City the periods between the epidemics are bridged over by cases of acute plague amongst the rats accompanied by a few cases in man. Knowing, then, the factors which determine the rise and fall of the epizootic amongst the rats, once the infection is present, we have a fairly complete conception of the seasonal prevalence of human plague in a city such as Bombay. In the case, however, of a large province, such as the Punjab, with scattered villages, the question of the annual recrudescence in the several villages is not so simply answered. For, it will be remembered that in the two Punjab villages of Dhand and Kasel, which were under close observation by the Commission for a whole year, no acute plague was found amongst either man or rats in the long interval between the epidemics. While this is so, a certain number of rats were caught alive, which, although apparently in good health, harboured living and virulent plague bacilli in chronic abscesses (see above, p. 335).

It is, however, to be noted, that after a careful study of this condition the Commission came to the conclusion that there was no direct evidence that "resolving" plague, as it occurs in the Punjab villages, possessed any significance in the seasonal recurrence amongst the rats of the infection in an acute form, nor was any evidence available which excluded this possibility. Since the observations were made in the Punjab this condition has been further carefully studied in Bombay, Poona and Belgaum, and, as we shall see elsewhere, the conclusion has been come to that the lesions denote rather a gradual and slow recovery from an acute condition than a definite chronic state. From these facts it is evident that we are still without certain knowledge of the cause of the beginning of plague in successive epidemics in the villages of India. It is this problem to which we now give attention.

On consideration of the problem two solutions seem possible. We shall for brevity designate these two hypotheses (1) recrudescence and (2) importation. Recrudescence implies that in each village each successive epidemic has its origin *in situ* from remnants left over from the previous epidemic no matter how far distant, or in other words, that dregs of the infection remain over from one epidemic and light up to start the next epidemic. It implies, therefore, that plague bacilli in each village survive the interval between two epidemics. From the work which we have already done it is certain that to fulfil this condition the bacillus must find a habitat in an animal body.

Importation, on the other hand, implies that the great majority of the villages became infected each epidemic by the bacilli being brought into them from without. As the bacillus must survive the non-epidemic season in some way or other, we have to postulate for this hypothesis that the infection in one or more villages or towns bridges over the off-season as scattered cases of acute rat plague and perhaps human plague or in some other way. From the centres or foci where this bridging over takes place the infection would spread out, when the conditions become suitable, exactly as must happen when a district becomes infected for the first time. The villages in which the infection is carried over the off-season might of course vary in different years.

The problem then which lies before us is to *determine the relative importance of recrudescence and importation in the spread of the epidemic through a district with scattered villages.*

We have now to state the methods which were adopted to find a solution for this problem. Three districts in the Punjab and in the United Provinces were selected, for the reasons that they had suffered from several consecutive epidemics of plague and that in the offices of the civil surgeons records of the deaths reported from the villages of the districts were available for analysis. A complete list of the villages in the district was prepared by the clerks in the civil surgeons office, men who knew the districts and who had kept the records. Against each village was shown its population and whether it had at any time been plague-infected or not. If it had at any time reported plague deaths, the following information for each epidemic was entered: the date of the first death, the date of the last death, and the total number of deaths. If the interval between the first and last death was more than three or four months, namely, the normal duration of a single epidemic in a village, the number of deaths month by month was recorded, so that any period of freedom from deaths might not escape notice. These data obtained from the district records have been analysed chiefly in the following directions.

First, maps have been prepared showing the infected villages month by month for several years from the first introduction of plague into the district. In these maps we are able to trace the spread of each epidemic and to compare the later epidemics with the first one, which we know must have spread by importation. The rise of each epidemic can also be graphically followed and can be correlated with the decline of the previous epidemic. The maps of Rohtak district are reproduced.

Secondly, the previous plague history of the villages infected in each epidemic has been determined with the object of obtaining data regarding the relative proportion of those which had never been infected before, of those which had been infected during the previous epidemic and of those which had escaped for either one or more epidemics.

Thirdly, in those villages in which the disease appeared first in each epidemic the previous history as regards plague infection, especially in the epidemic just passed, has been traced.

Fourthly, in some districts only a small number of villages were infected in one or more of the epidemic years. We have traced the fate of all these villages in the following year in which the epidemic was widespread and involved a very large number of the villages of the district.

Fifthly, we have followed in detail the future history as regards plague infection of villages infected at the end of each epidemic, so as to ascertain in what proportion of them deaths were reported early in the next epidemic.

Finally, we have investigated the question whether plague tends to recur in villages in successive epidemics, and have also analysed the relation of the size of a village to its liability to become infected.

In considering the results obtained in this way, it is necessary to bear in mind the limited accuracy of the records. In particular they tell us nothing of rat plague which is not accompanied by obvious human plague. The fact for example that a village has not returned a plague death is not an absolute proof that there has not been a small rat epidemic, accompanied perhaps by a few mild human cases. It is therefore possible that the first year of infection of any village is recorded as being later than it really was. It is, however, impossible to estimate the weight of these possibilities. Considering the intimate domestic relationship of rats and human beings in the Punjab villages, it seems to us unlikely that rat plague could often exist without being indicated by human plague; where this is known to have occurred, the conditions have been very different. In any case, we have no choice but to deal with the data as they stand: we have little doubt that they give a substantially accurate picture of the incidence of plague infection in the villages concerned.

B. *Districts in which observations were made.*

(1) *Rohtak.* Rohtak is a small district in the south of the Punjab. It is about 64 miles long by 43 miles broad and has an area of about 2800 square miles. There are no natural boundaries such as rivers, etc., so that there is free intercourse on all sides. The total population is about 620,822 which gives a density of 0·3 per acre.

There are 499 municipal towns and villages, none of which, however, are very large. There are only three municipal towns, namely, Rohtak (pop. 20,024) and Beri (pop. 9723) in the Rohtak subdivision, and Jhaggar (pop. 12,227) in the subdivision of the same name. The position of these towns is shown on the maps. All other settlements are villages with a population of less than 8000. The average population per village is 1244.

The district is divided for administrative purposes into four subdivisions or tehsils; Gohana in the north, Jhaggar in the south, and two in the centre, namely Rohtak to the west and Sampla to the east. A few details concerning the distribution of the population in the different tehsils are given in Table I. The point in this table to which we would especially draw attention is the relatively small average population of the villages in the Jhaggar tehsil. While the total population of this tehsil is the smallest of all, it is divided up amongst the greatest number of villages, so that the average population per village is from ½ to ⅓ that of the other tehsils.

Plague was introduced into the Rohtak district in the winter of 1903—04, the first deaths being reported in the Rohtak tehsil in November 1903. Up to July 1907 there were four epidemics, two slight ones alternating with two severe ones (vide Table II). Of the 499 villages in the district 145 were not infected in any of the epidemics, leaving 354 which returned deaths in one or more of the epidemics.

The seasonal prevalence of the epidemics was well marked (Table III). The deaths began to increase each year in the autumn (September—October) or early winter (November—January). The increase continued until a maximum was reached in April or May, after which there was a sudden fall in June. During July and August only a few villages returned deaths or sometimes none at all.

(2) *Mozuffarnagar* is a district in the north of the United Provinces, not far from the southern boundary of the Punjab. It is bounded on the west by the river Jumna, while all along the east side runs

the sacred Ganges. On the north and south there are no natural boundaries. The total population of the district is about 917,896. There are 973 inhabited municipal towns and villages, so that the average population per village is 944. There are only three towns with a population of more than 10,000, namely Mozuffarnagar (23,444), Kairana (19,304), and Kandla (11,563). All the other villages have less than 9000 inhabitants each.

The division of the district into thanas has been adopted for our present purpose. There are 18 of these subdivisions in the district.

Plague was first introduced into the Mozuffarnagar district in January 1902. Up to the summer of that year only four deaths were returned, so that it is improbable that there was any indigenous infection in that season.

In November 1902 the epidemic began in earnest and from then up to 1907 there have been five epidemics, the data referring to which are given on Table IV.

Of the 973 villages in the district, 334 have been at no time infected leaving 639 which have reported deaths in one or more of the epidemics. The seasonal prevalence of the epidemics has been well marked. The deaths began to increase each year in the autumn (September—October) or early winter (November—January). The maximum was reached in April, after which there was a fall until by the end of June the epidemics had practically ceased (Table V).

(3) *Amritsar.* Amritsar is a moderate sized district in the centre of the Punjab lying a short distance from the foot of the Himalayas. It has an area of 1601 square miles, the total population being 1,039,620 which gives a density of about 1 per acre.

Along the south-east limits of the district runs the Beas river, while on the north-west the Ravi separates it from the Sialkot district. There are no natural boundaries along the other two sides of the district, which is roughly square in shape. The main line of railway runs practically from east to west and the district is further intersected by many canals, the general direction of which is from north-east to south-west. There are four municipal towns and 1058 inhabited villages. Of the former Amritsar, the chief town of the district, is by far the largest, its population being 162,429. The three other municipal towns Tarn-Tarn, Majitha and Jandiala have a population between 4428 and 7877. Only very few of the villages have more than 4000 inhabitants. The average population per village, including the municipal towns, is 978. The district is divided into three tehsils, namely Ajnala in the north, Amritsar in the centre, and Tarn-Tarn in the south.

Plague deaths were first reported from the Amritsar district in February 1902. This epidemic, beginning late in the plague season, had only affected 62 villages when the hot weather limited its further spread. Succeeding this mild outbreak there have been five more or less severe epidemics (Table VI), the mildest being that of 1905—06 in the course of which about a quarter of the villages in the district became infected. The other four epidemics have all been severe, in each instance about half or more of the villages returning plague deaths. There were, therefore, three consecutive seasons, 1902—03, 1903—04, and 1904—05, during which the district was badly infected, a condition of affairs somewhat different to that which has been described as occurring in the Rohtak district, in which two mild epidemics alternated with two severe ones.

Of the 1062 towns and villages in the district 907 have in the course of one or more of the six epidemics returned plague deaths, leaving 155 which have not at any time been infected.

As was observed in the other two districts the seasonal prevalence had been well marked (Table VII). Each epidemic, beginning in the autumn or early winter, has gradually gained strength until the height was reached in April or May. After May the decline has been rapid, so that by the end of July very few villages remained infected; that is to say, returned plague deaths. Finally, it is important to note that since the disease was first introduced into the district in February 1902 until the end of July 1907, there have only been two months, namely, September and October 1903, during which one or more villages did not report plague deaths. In fact, this large area has at no time even in the off-season been free from human plague and as a corollary acute rat plague must always have been present in the district.

Analysis of the data obtained.

II. METHOD OF SPREAD OF THE EPIDEMICS IN EACH DISTRICT AS JUDGED FROM THE MAPS.

The maps, as we have stated, were prepared from the records of plague deaths which were available in the district plague offices. It is to be noted that the criterion of infection is the occurrence of a death or deaths from plague so that when a village is spoken of as being infected it means that one or more deaths from plague have been reported among the population.

A. *Rohtak District.*

Maps I—XLVIII[1].

(*a*) *Epidemic of* 1903—04. Deaths began to be returned in November 1903 from two separate villages in the extreme west of Rohtak tehsil and from Beri town situated to the south of the district.

In January 1904 the pattern suggests a slight spread out from Beri and there appears a fresh infection in the extreme south of the Jhaggar tehsil. The maps of the following months show a well marked spreading out from the Beri centre and also an increase of infected villages round about those which first returned deaths in the west and south of the district. The town of Rohtak, the largest in the district, did not become infected till late in the epidemic, namely in April, and there was hardly any spread to the northern portion of the district, the Gohana tehsil remaining practically free. In June the epidemic subsided and in July only one village, to the west of Beri, still reported deaths.

It is important to keep in mind the pattern of these maps. The villages were all infected for the first time, so that the origin of the infection in each case was presumably due to importation. The pattern as we have seen suggests a spreading out as the epidemic progresses from two if not from three foci, into which the disease was introduced at the beginning of the epidemic.

(*b*) *Epidemic of* 1904—05. This epidemic began in August 1904, that is to say, there was no month between it and the end of the previous epidemic in which plague deaths were not reported from the district. In August two villages returned deaths, one in the Rohtak tehsil quite close to Beri and the other in the south of Jhaggar. It will be remembered that in 1903—04 both these localities had been infected. Further, one of the villages, namely, that in Rohtak, had itself been infected as late as May: the other village had not previously reported deaths.

In September there appears to be a slight spread out from the Beri centre and a new centre crops up in the Gohana tehsil. This latter village, although not itself previously infected, is situated next to the only village in this tehsil which was infected in the previous epidemic, and in which deaths took place as late as June. It is also worthy of note that the three villages in the neighbourhood of the Beri centre had all been infected late in the last epidemic, two as late as June and the third one up to May.

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[1] In the months for which no maps are given there was no plague in the district. The circles represent epidemics with less than five plague deaths.

The October map shows a slight increase of the number of infected villages in the neighbourhood of Beri. Rohtak town is now also infected.

In November and December more infected villages appear around Beri and also in the neighbourhood of the centres established in August in the south of Jhaggar tehsil and in September in the north in Gohana.

It is evident, therefore, that by the end of December the infection had become fairly widespread. The subsequent maps show a gradual thickening of infected villages all over the district, in the January map the spread being traceable from the centres already established, especially around Rohtak town. In June the epidemic markedly subsided, the villages then infected being chiefly situated in the north-eastern portion of the district. In July only a single village returned deaths.

(*c*) *Epidemic of* 1905—06. During August and September 1905 the district was apparently free from plague as no villages returned any deaths.

In October two villages showed infection, one in the north of Gohana tehsil and the other in the south-east of Sampla. The former had returned deaths as late as June 1905 and the latter a few deaths in May 1905. Further, in June both were surrounded by infected villages.

In November and December there is little change, only one infected village in the north of Gohana being added in December. This village was also infected the previous epidemic as late as June. From now onwards the main feature of the maps is the gradual increase of infected villages in the north of Gohana, round about the villages which first returned deaths. A very few scattered villages, chiefly in Rohtak tehsil, become infected, but there is no appearance of a spread out from any centre except from the one in the north of Gohana. Here the infection lingered up to June, in which month four villages in this tehsil were still returning deaths.

In July no deaths were reported from anywhere in the district.

It is to be remembered that the epidemic of 1905—06 was a slight one, only 30 villages being reported infected.

(*d*) *Epidemic of* 1906—07. In August 1906 a single village began to return plague deaths. It was situated in the north of the Rohtak tehsil, but only a few miles to the south of the four villages which had reported deaths in June 1906. Further this village had itself been infected the previous epidemic, the last death being reported on the 27th May.

In September three more villages were returned infected: two in Gohana tehsil to the north of the village infected in August, and the third in the extreme east of the Sampla tehsil. Of these three villages only one of them had been infected in 1905—06, the last death taking place on 10th June, 1906.

In October again three new villages reported cases, two in Gohana and one in Sampla tehsil. Only one of these villages had been infected in the previous epidemic.

During the next two months a few more villages in the northern portion of the district became infected making 11 villages which up to the end of 1906 returned deaths. It is important to note that of these 11 villages only four had been infected in the previous epidemic, the others having been apparently free from plague in every instance for at least 15 months. Further, up to the end of December, the infection was entirely confined to the north and east, especially the north, portions of the district.

The remaining maps of this epidemic show a gradual thickening of the infected villages in the north-east with a later incursion into the centre and over the centre into the west. Rohtak tehsil in the west is infected late, namely March, April and May, while Jhaggar in the south is hardly infected at all. In this tehsil up to the end of March only four villages had reported deaths, not one of which had been infected in the previous epidemic. The pattern in short suggests a spread out from the centres established early in the north and east.

B. *Mozuffarnagar District.*

The first plague death in the district was reported from Mozuffarnagar town in January 1902. This was probably an imported case as nothing further happened.

In April of the same year a village, Biralsi, in Charthawal thana reported three deaths. As there was no further sign of infection in this place till December 1904, these also were probably not indigenous cases.

No more deaths were returned until November 1902, when the first epidemic began.

(*a*) *Epidemic of* 1902—03. This epidemic was a small one, only 25 villages becoming infected. It was entirely confined to the south-east half of the district suggesting that the invasion had taken place from this direction. It is interesting to note in passing that the railway passes from south to north through this portion of the district.

With the exception of the thana of Budhana, in which several villages close together returned deaths, the patterns of the maps do not suggest a spread out from any definite centres, the villages which became infected being all widely separated from one another. We would especially draw attention to this springing up of the infection in villages at a distance from one another. This fact is of the greatest importance as, it is to be remembered, we are now dealing with the first epidemic in the district when every village presumably owed its infection to importation.

(*b*) *Epidemic of* 1903—04. The epidemic of 1902—03 came to an end in the month of June 1903. During July and August of this year no villages reported plague deaths.

The epidemic of 1903—04 began in two towns, both of which had been infected late in the previous outbreak and showed an interval of only three months free from deaths.

The patterns of the maps suggest a spread out from these centres and at the same time the development of fresh infections further afield, which villages again become the centres of spread. It is to be noted that the great majority of the villages which reported deaths in this epidemic had never been infected before. Thus of 130 villages which returned cases, only nine of them had been infected in 1902—03, so that in the case of at least 121 (93 °/₀) villages the origin of the outbreak may be attributed to importation.

(*c*) *Epidemic of* 1904—05. Taking this epidemic to have begun in July 1904, there was no interval of freedom between it and the previous outbreak.

Tijalhera, a village in Purkazi thana, became infected in April 1904 and reported deaths right through the off-season till 10th September, 1904; Bebra in Bhopa thana became infected in May 1904 and continued without interruption to return deaths until 7th October, 1904. In July, as well as these two villages, two others close together in Charthawal thana each reported two deaths. They had never been infected before.

The bridge over between this epidemic and that of 1903—04 is therefore clear and easily traceable. The patterns of the maps, taking into consideration the facts that the epidemic was a severe one and that it had its origin probably from several centres left over from the previous epidemic, do not differ from those of the first outbreaks. They even suggest a spread out from the centres which showed infection early in the epidemic.

(*d*) *Epidemic of* 1905—06. At the end of the epidemic of 1904—05, namely, in May and June 1905, infected villages were plentiful and were scattered over practically the whole district. It is not surprising, therefore, to find the present outbreak springing up in places widely separated from each other and also in villages which had not been infected in 1904—05. This epidemic was a small one, only 69 villages being infected. Taking into account that the infection was probably carried over from the epidemic of 1904—05 in several centres the patterns of the maps in no way differ from those of the first two epidemics.

(*e*) *Epidemic of* 1906—07. One death in each of the months of July and August 1906 was reported from the town of Kandla in the south-west corner of the district. This town had returned many deaths as late as May 1906. The epidemic proper, however, began in several centres at a distance from one another (vide October and November maps). The patterns of the maps suggest, as in the first epidemics, a spread out in the immediate neighbourhood of these centres and at the same time a spread to villages at a distance. The important thing to note is that the patterns are in no way different from those of the maps of the early epidemics, in which, as we have remarked, importation may be considered to have been the cause of the origin of the outbreaks in the villages.

C. *Amritsar District.*

(*a*) *Epidemic of* 1901—02. This epidemic, which was a small one, began in Amritsar town in February 1902.

The patterns of the map do not suggest at the beginning a regular centrifugal spread from Amritsar town. During April and May there was, however, a marked increase around Amritsar and also around some of the villages which were infected in March.

It is worthy of note that the northern tehsil of Ajnala remained practically free during the whole of this epidemic.

(*b*) *Epidemic of* 1902—03. Amritsar City was the only place which reported plague deaths throughout the off season. The patterns of the maps suggest a spread out from Amritsar itself and from the places which first reported deaths in October 1902.

Infected villages were at first confined to the centre of the district and only later on in the epidemic did the outlying north-west and south-east portions become infected. As importation must have been the all-important factor in the spread of this epidemic the patterns of the maps should be carefully studied. 32 villages were still reporting deaths as late as July 1903.

(*c*) *Epidemic of* 1903—04. The place in which the infection remained over from the epidemic of 1902—03 to that of 1903—04 cannot be clearly demonstrated. Plague deaths were reported in Amritsar City till the end of August 1903 and appeared again in November just to the north of this City. There were two months during which no plague deaths were reported.

The patterns of the maps suggest a spread out from centres established by January 1904. They resemble those of the previous epidemic.

(*d*) *Epidemic of* 1904—05. In August 1904 a village in the north of Amritsar tehsil returned two deaths. It had not been infected during the epidemic of 1903—04, the last death having occurred 13 months previously. In September four more villages returned deaths. Three of these villages had never been infected before while the fourth had enjoyed a free interval of 15 months. It seems likely that into every one of these villages the infection was imported either from one of the others or from places infected at the end of the previous epidemic.

The patterns of the maps in no way differ from those of the previous epidemics. They suggest a spread out from villages which became infected early in the epidemic, namely, by October 1904.

(*e*) *Epidemic of* 1905—06. Amritsar town had no interval of freedom between the epidemic of 1904—05 and that of 1905—06. It was the only place which reported deaths during the months of August, September and October 1905. The patterns of the subsequent maps strongly suggest a spread out from three centres, namely, Amritsar town and centres on the north-east and east of Amritsar tehsil.

(*f*) *Epidemic of* 1906—07. Amritsar City continued to report deaths up till 25 August, 1906, bridging over a considerable portion of the off season. It was the only place which returned deaths in August.

After this date no more deaths occurred in Amritsar town until January 1907.

The epidemic proper began in two centres, one in the north-east of the district in Ajnala tehsil and the other in the east corner. It was well advanced before Amritsar City began to return deaths. The patterns of the maps suggest a spread out from the places infected in the early months of the epidemic and in no way differ from those of previous epidemics.

D. *Summary.*

In the three districts of Rohtak, Mozuffarnagar and Amritsar the methods of spread from village to village or from four to six consecutive epidemics, beginning with the first one, have been traced on monthly maps.

The patterns of the maps of the later epidemics are of the same type as those of the first two epidemics, both of which may be presumed to have owed their spread to importation and not to recrudescence. They, as a rule, suggest a gradual spreading out from centres or foci which become established early in the epidemic. These centres of origin may not be the same in different years. In Amritsar district in some of the epidemics Amritsar City seemed to be the centre from which the infection spread, while in others it was not infected till late on in the outbreak.

III. PREVIOUS PLAGUE HISTORY OF THE VILLAGES INFECTED IN EACH EPIDEMIC.

We propose now to analyse in each district the previous plague history of the villages infected in each epidemic with the object of obtaining data regarding the relative proportion of those which had never been infected, of those which had been infected the epidemic previous, and of those which had escaped for either one or more epidemics.

The analysis of the data available is contained in Tables VIII—X. These tables require little or no explanation and all lead to the same conclusions.

In the case of the first two epidemics, as 85 to 100 p.c. of the villages infected had never before suffered from plague, importation must have been the chief factor in the origin of the outbreak in each village. In the case of the epidemics either small or large following on severe ones, *e.g.* the third epidemic in Rohtak district, the fourth epidemic in Mozuffarnagar district and the third, fourth, and fifth, epidemics in Amritsar district, from 50 to 85 p.c. of the villages had been infected in the previous year. The present data in these instances, therefore, do not allow us to come to any definite conclusion as regards the relative importance of importation and recrudescence.

In all the other epidemics, however, that is to say, in those years in which the previous epidemic was slight or moderate in extent, the percentage of villages which had shown infection during any period less than 18 months is so small that unless it is possible for the infection to lie dormant over at least one epidemic season, that is to say for 18 months or longer, we must conclude that importation played the most important part in the spread of these outbreaks[1]. In addition to the data collated in these tables, we have to consider that importation is at least as likely to occur into villages which have been previously infected as into villages which have never been infected.

IV. PREVIOUS PLAGUE HISTORY OF VILLAGES INFECTED AT THE BEGINNING OF EACH EPIDEMIC.

We have already in passing drawn attention to the fact in that certain instances villages, which began to return plague deaths at the very beginning of an epidemic season, had been infected late in the previous epidemic with perhaps no interval at all or one of only two months free from deaths. This observation suggests that in these villages the infection survived probably as acute plague in the rat. We propose now to consider more fully for each district the data referring to this question, taking the villages first infected in each epidemic and inquiring into their previous plague history. We shall by this means obtain an idea of the proportion of villages reporting deaths early in an epidemic which probably harboured the infection from one epidemic to another as acute rat plague. These data are contained for Rohtak district in Tables XI—XV, for Mozuffarnagar district in Tables XVI—XX, and for Amritsar district in Tables XXI—XXVI. Tables XV, XX, and XXVI show for each district the data referring to villages in which the infection was very probably carried over from one epidemic to another as acute rat plague.

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[1] It is possible that a rat epidemic might occur without a human epidemic. Owing to the intimate relation between men and rats in these villages, this is very unlikely : the conditions elsewhere are quite different.

The following remarks on these several tables will lead to their better understanding.

A. *Rohtak District.*

(*a*) *Epidemic of* 1903—04. (Table XI.) As this was the first introduction of plague into the district none of these villages had ever been infected before. It is, however, interesting to note that between November 1903 and February 1904 ten villages had returned plague deaths.

(*b*) *Epidemic of* 1904—05. (Table XII.) This was a very severe epidemic, following on one in which only a small proportion of the villages had been infected.

From August to November 1904 inclusive 15 villages returned plague deaths and of these nine had been infected during the previous epidemic. When we consider that only 57 villages in the whole district had been infected in 1903—04, this high proportion is strong evidence of the infection having been carried on in some at least of these villages. This presumption is supported from the fact that the interval during which no plague deaths were returned from some of the villages was a comparatively short one. Thus out of the nine villages three had a free interval of only two months, two of three months, two of four months and two of five and six months each.

In December six new villages reported deaths: none of these had been previously infected.

(*c*) *Epidemic of* 1905—06. (Table XIII.) This epidemic it will be remembered followed a very severe and widespread outbreak but was itself mild and limited in extent, only 30 villages returning deaths.

In October 1905 two villages reported deaths. Both of these villages had been infected in the previous epidemic and had been free from deaths for three or four months respectively.

In December and January four other villages began to return deaths. These four villages had also been infected in 1903—04 but had enjoyed a free interval of from five to seven months.

(*d*) *Epidemic of* 1906—07. (Table XIV.) In the epidemic of 1906—07 half the villages of the district were infected, a contrast to the previous one in which only 30 villages reported deaths. During August and September already four villages were returning deaths.

Two of these villages had been infected in the previous epidemic and had enjoyed only two months in which no deaths occurred. The other two villages had not been infected since 1904—05 and in both instances there were 15 months during which no plague deaths had been reported.

During October, November and December, several more villages returned deaths. Of these only two had been infected in the epidemic of 1905—06 and the interval of apparent freedom was three and five months respectively.

Thus during the early months of this outbreak out of 11 villages which reported deaths only four had been infected in the previous epidemic, the intervals of freedom being in two instances two months, in a third three months, and five months in the fourth. In the case of the other seven villages no plague deaths had been reported for from 15 to 21 months.

B. *Mozuffarnagar District.*

(*a*) *Epidemic of* 1903—04. (Table XVI.) During September 1903 to January 1904 inclusive 16 villages began to report deaths from plague. Of these villages 13 had not been infected before, and three had suffered during the previous epidemic; one of these, however, had returned only one death in the epidemic of 1902—03.

In the case of both the other two villages there was an interval of three months in which no plague deaths were reported. It is also important to note that both these villages began to report cases very early in the epidemic, namely, in October 1903.

(*b*) *Epidemic of* 1904—05. (Table XVII.) The epidemic of 1904—05 was a severe one following on a comparatively mild one.

During the first four months (July—October) plague deaths occurred in 26 villages. In two of these villages the bridge over was complete, that is to say, there was no interval during which the villages were free from deaths between the beginning of this and the end of the last epidemic. One village was infected from April to September and the other from May to October inclusive. A third village, which returned one death in August but after this date none till October, had been badly infected as late as June. It is probable, therefore, that the infection was present as acute rat plague during this period, namely, June—October. A fourth village had an interval of two months, July and August, free from plague deaths. In the case of other four villages which evidently had comparatively short intervals of freedom, three to five months, it is doubtful whether they were really infected or not in the outbreak of 1903—04, as each then returned only one or two deaths.

Of the remaining 18 villages, which began to return plague deaths early in this epidemic, 15 had never been infected before, and the other three had enjoyed a free interval of from 17 to 18 months, that is to say, had not been infected during the previous epidemic.

From this short account it is seen that only in a very few of the villages infected at the beginning of this epidemic was it reasonable to suppose that the origin of the disease was due to recrudescence from remnants left over from the previous epidemic. In these few villages, however, and especially in the two which had no free interval, it is likely that the infection persisted throughout the off season in an acute form amongst the rats, constituting a complete bridging over of one epidemic to the other.

(*c*) *Epidemic of* 1905—06. (Table XVIII.) This was a very small outbreak following on a very severe one.

We have analysed the previous plague history of 19 villages which began to report deaths at the beginning of the epidemic, that is to say, during August 05—February 06. Of these villages six had never been infected before; one had been infected in the 1903—04 epidemic and had enjoyed an interval of freedom for 19 months; the remaining 12 villages had been infected during the previous epidemic. But in the case of four of these villages there was only one or two deaths reported in the epidemic of 1904—05, and in three others there was only one death in the epidemic of 1905—06, so that we are justified in concluding that in none of these seven villages was it likely that the infection had survived from one epidemic to the other. That leaves us with five villages which were definitely infected in both epidemics. The intervals during which no deaths were reported in these villages was 2, 5, 7, 11 and 12 months respectively.

(*d*) *Epidemic of* 1906—07. (Table XIX.) This was a very severe epidemic, affecting as it did more than half the villages of the district. It will be remembered that it followed on a very mild outbreak.

We have traced the previous plague history of the 45 villages which were the first to report deaths, that is during the period from July to December 1906 inclusive.

Twelve of these villages had never been infected in any previous epidemics; two had not returned any deaths since the epidemic of

1903—04, having enjoyed a free interval of 29 and 30 months respectively: twenty had been last infected in the severe outbreak of 1904—05, having escaped in the epidemic of 1905—06. The interval of freedom from deaths in these villages varied from 17 to 22 months. In none of these villages therefore, is it at all likely that the infection survived from one epidemic to the other. We are now left to analyse the data in the case of the remaining 11 villages which were infected in the two consecutive epidemics of 1905—06, and 1906—07. From the table it will be seen that in the case of three of these villages there was only one death in one or other of the epidemics so that the infection was probably not indigenous. In the remaining eight villages the interval during which no deaths were reported varied from one to six months, and in all these, there is the possibility of the infection bridging over the epidemics as acute plague in the rat. Amongst these villages Kandla, a town with a population of over 11,000, has a most interesting history. It was infected badly in the epidemic of 1905—06, the last death being reported on 25th May, 1906. In June no deaths were reported, but in each of the months of July and August one death came to light. During September and October again no deaths were returned as due to plague, but in November between the 8th and 14th seven deaths were reported as plague. From this latter date until the 24th February, 1907, the epidemic appeared to be entirely in abeyance. It then started again and between 24th February and 4th July, 1907, 600 deaths took place.

Such a history as this appears to us strongly in favour of the supposition that in this town acute plague amongst the rats accompanied by a few human cases was present during the whole off season and bridged over the interval between the two epidemics.

C. *Amritsar District.*

(*a*) *Epidemic of* 1902—03. (Table XXI.) Amritsar City had no interval free from deaths between the epidemic of 1901—02 and that of 1902—03. In July 1902 there were three, in August 14 and in September 47 deaths. It is certain, therefore, that acute rat plague was present in the city throughout the off season.

Of the 45 villages which began to report deaths early in this epidemic, namely, in October and November, only seven had been previously infected. They had been free from deaths for from three to four months. It is probable, therefore, that in some of these villages also acute rat plague bridged over the two epidemics.

(*b*) *Epidemic of* 1903—04. (Table XXII.) In Amritsar City the epidemic of 1902—03 was continued into August, the last death being reported on 26. VIII. 03. No more deaths were returned until January 1904 so that the bridge over of the two epidemics as acute rat plague cannot be completely demonstrated.

Up to the end of December 1903 only two villages returned plague deaths. One of these had reported a single death twelve months before while the other had enjoyed a free interval for six months.

During January 1904, 20 villages began to return deaths. Three of these had never been infected before, one had been last infected in the epidemic of 1901—02, while the remainder had reported deaths in the previous epidemic. The shortest interval of freedom, however, which any of these villages enjoyed was six months, while some of them had been free for as long as 10 to 11 months.

(*c*) *Epidemic of* 1904—05. (Table XXIII.) In August and September 1904 five villages began to return plague deaths. Three of them had never been infected before, while the other two had had free intervals of 13 and 15 months respectively. In not one of these villages, therefore, is it probable that the epidemics were bridged over by acute plague in the rat.

During October and November, 1904, 37 villages became infected. Of these villages eight had never been infected in any of the previous epidemics and 13 had been for 14 months or longer free from deaths. That leaves us with 16 villages which had reported plague deaths in the previous epidemic of 1903—04. Three of these villages had a free interval of three months, seven had four months, five had five months and one six months.

(*d*) *Epidemic of* 1905—06. (Table XXIV.) Amritsar City was still reporting plague deaths in August 1905; no free interval occurred between the end of the last epidemic and the beginning of that of 1905—06. From the beginning of August till the end of the year in this city 40 deaths were reported as being due to plague. There can be no doubt, therefore, that acute rat plague must have been present in Amritsar City throughout the whole of the off season of 1905.

During August, September and October 1905, no other place except Amritsar City reported deaths. During November and December 1905, three more villages were recognised as being infected. Only one of these had been infected in the previous epidemic, the last death having been reported in March 1905, *i.e.* there was a free interval of at least seven months.

During January and February 1906, 22 villages began to report plague deaths. As will be seen from the Table everyone of these villages had been infected in some previous epidemic, 19 in the severe epidemic of 1904—05 and the other three in that of 1903—04. The shortest interval of freedom from deaths enjoyed by any of the villages infected in 1904—05 was five months and the longest 10 months.

(*e*) *Epidemic of* 1906—07. (Table XXV.) In August 1906 Amritsar City still continued to report deaths from plague. The last death occurred on 25. VIII. 06, and from that date till January 1907 the city was apparently free from the disease, that is to say, no deaths were returned.

In September two villages began to report. One of these villages had not been infected since December 1904, while the other had only been two months free from deaths.

In October three more villages were reported to be infected. One of these villages had never been infected before while the other two had been free from deaths for 15 and 40 months respectively.

During November and December 1906 38 new villages began to return plague deaths. Of these villages three had never been infected before, 23 had been infected in some previous epidemic but not in that of 1905—06, while the remaining 12 had returned deaths during the last epidemic, the interval of freedom enjoyed being from 4—6 months.

Lastly in Tables XV, XX and XXVI we have for each district collected together from the various epidemics the data referring to those villages in which, judging from the short period of freedom from plague deaths, the infection was probably carried over from one epidemic to the next one as acute rat plague. It would appear then that at the beginning of each epidemic amongst the first villages to report plague deaths are some in which deaths have occurred late in the previous epidemic and that there are instances in which there may be no interval of freedom at all. It is to be remembered that the data under analysis only refer to plague deaths not to cases which recover, and that it is more than likely that both cases and deaths occurring in small number in the off season would be concealed, the deaths being returned as due to some other cause. It is of course possible that the epizootic amongst the rats might continue without any plague cases in the human population, held in check by the unfavourable conditions to which we have drawn attention in another paper (*Journal of Hygiene*, vol. VIII. p. 266), namely, a mean temperature above 86° F. and a paucity of rat fleas. If, therefore, all human plague cases had come within our ken and especially if the rat population could have been examined as was done in Bombay it is probable that the bridging over the off season by acute plague in the rat would have been more clearly demonstrated and in more villages than we have been able to do with the data at our disposal.

Two points of interest and importance require mention before leaving this part of our subject. First, it is seen from the tables that the villages in which the epidemics are bridged over are of large size with a population considerably greater than that of the average village of the district. It is also seen that with few exceptions, *e.g.* Amritsar town, the villages in which apparently the infection survives the off season vary from year to year.

D. *Summary.*

Villages which return plague deaths early in an epidemic are in some instances those which were infected late in the previous epidemic. There may be no interval free from deaths or it may be as short as one to three months. In the great majority, however, of early infected villages the interval of freedom is very much longer, suggesting a fresh importation of the infection.

The villages in which one epidemic is bridged over from the previous one are as a rule of large size. They also, with the exception of the large towns such as Amritsar, vary from year to year.

V. FUTURE PLAGUE HISTORY OF THE VILLAGES INFECTED IN THE COURSE OF THE MILDER EPIDEMICS.

In all three districts some of the epidemics were very mild, affecting only a comparatively small number of the total villages. It was a simple matter, therefore, to follow up the history of all these villages and to put into tabular form the data obtained. This has been done for two epidemics in two of the districts, namely Rohtak and Mozuffarnagar.

An analysis of these data throws light on the question of the bridging over of the epidemics. The data and their analysis are contained in the following tables:—

(1) Rohtak district—Tables XXVII—XXXII.
(2) Mozuffarnagar district—Tables XXXIII—XXXVIII.

To these tables the following remarks apply.

A. *Rohtak District.*

(*a*) *Epidemic of* 1903—04. From Table XXVII it is seen that of the 57 villages which were infected during this epidemic six were not again infected and seven did not return any more deaths until the epidemic of 1906—07, that is to say, nearly two years afterwards. Further 13 villages reported less than three deaths during the epidemic under consideration, suggesting the probability that they were not truly infected but that the cases contracted their infection elsewhere. We are, therefore, left with 31 villages, which, infected in the epidemic of 1903—04, were again infected in 1904—05. It is possible, therefore, that in every one of these villages the second epidemic had its origin in remnants left over from the first one. When, however, we consider that more than half the villages of the district were infected in 1904—05 and that the great majority of them presumably owed the origin of infection to importation, it is justifiable to assume that a certain proportion of these 31 villages were also infected by importation. This assumption receives material support from an analysis of the data, which refer to the period during the second epidemic at which these villages first returned deaths (Table XXVIII) and which show the number of months during which they were free from plague deaths (Table XXIX).

From Table XXVIII it is seen that in only nine out of the 31 villages were plague deaths returned at all early in the epidemic of 1904—05, that is to say, before January 1905. Further, from Table XXIX it is seen that only in the case of seven of the villages was the free interval less than five months.

(*b*) *Epidemic of* 1905—06. We have analysed the future history of the villages infected during this epidemic in the same manner as was done with those of the 1903—04 epidemic. The data are set forth in Table XXX.

In all 30 villages returned plague deaths; four of these were not infected in the epidemic of 1906—07 and six had less than five deaths, and these we have taken to be imported cases, that is to say, received their infection elsewhere. We are, therefore, left with 20 villages which were definitely infected in both epidemics and in the case of all of which it might be argued that the infection in 1906—07 originated from remnants left over from 1905—06. But as happened in the epidemics of 1903—04 and 1904—05, very few, namely, four, of these 20 villages returned deaths early in the second epidemic (Table XXXI) and the interval free from deaths was five months or less only in the case of four of the villages (Table XXXII).

B. *Mozuffarnagar District.*

(*a*) *Epidemic of* 1902—03. In Table XXXIII are set forth the details as regards plague infection of the 25 villages which reported plague deaths during this epidemic. From this table it is seen that 11 of the villages returned only one or at most two deaths, so that it is more than probable that these cases were imported and that there was no indigenous plague. They are, therefore, left out of account. Of the remaining 14 villages three were never again infected. The analysis of the data as regards the future plague history of the remaining 11 villages is set forth in Tables XXXIV and XXXV. Six of the villages were again infected in the next epidemic but only two of these six returned cases at all early in the course of the outbreak, having enjoyed a free interval of three months or less. The other four became infected well on in the epidemic at a time when many other villages were reporting deaths. We are left with five villages which, having escaped in the epidemic of 1903—04, were infected in some subsequent outbreak. They enjoyed free intervals of from 16 to 20 months.

(*b*) *Epidemic of* 1905—06. This was a more widespread epidemic than that of 1902—03, 69 villages in all reporting plague deaths. It was followed by a very severe outbreak, the last of which we take any cognizance.

The data concerning the infected villages are set forth on Table XXXVI. From an analysis of this table it is found that seven of the villages were not infected in the following epidemic leaving 62 which reported deaths in both epidemics. But in the case of nine of these villages there was only one death reported in one or other of the epidemics, so that we are left with 53 villages which can be said to have been definitely infected in both epidemics. Further analysis shows us that the great majority of these villages began to return deaths well on in the epidemic, when a great many other villages were already infected and that only a small proportion had a short interval of freedom or reported deaths early in the epidemic (Tables XXXVII and XXXVIII).

C. *Summary.*

A relatively small number of villages in the Rohtak district were infected in the epidemics of 1903—04 and 1905—06, and in the Mozuffarnagar district in the outbreaks of 1902—03 and 1905—06. Even when the majority of the villages which returned deaths in these epidemics were again infected in the following epidemic, analysis of the data shows that in the great majority of these villages the infection did not take place until late in the epidemic at a time when many other villages, not infected in the mild epidemic, were returning deaths. Importation, therefore, is at least equally likely to have been the origin of the second outbreak as recrudescence.

VI. FUTURE PLAGUE HISTORY OF VILLAGES INFECTED AT THE END OF EACH EPIDEMIC.

We have already seen that the villages infected at the beginning of an epidemic are sometimes, but comparatively rarely, those which have returned deaths at the end of the previous epidemic and that in some instances there may be no period of freedom from plague deaths, or that the free interval may be as short as from one to three months. We propose in this section to inquire into the future plague history of those villages which reported deaths at the end of the various epidemics in the different districts. We hope thus to obtain some idea of the proportion of late infected villages which are infected early in the next epidemic. The importance, also, of such an inquiry from a prophylactic point of view is evident. The crude data as regards the villages in Rohtak district are set forth in Table XXXIX, in Mozuffarnagar district in Table XL, and in Amritsar district in Table XLI. The analysis of these data is contained in Tables XLII—XLIV.

A. *Rohtak District.*

(*a*) *Villages infected at end of the epidemic of* 1903—04. In June 1904 nine villages had their last plague death, while in July the infection apparently remained only in one.

From all of these ten villages deaths were reported in the next epidemic, which it will be remembered was very severe and widespread. When, however, we come to consider the interval during which these ten villages returned no deaths (Table XLII) we see that the majority of them did not again show infection till late on in the 1904—05 epidemic, which by that time was widespread and affecting many villages. Only three of the ten villages had a free interval of less than five months.

(*b*) *Villages infected at end of the epidemic of* 1904—05. This was a severe epidemic and a considerable number of villages still reported deaths towards the end, namely 36 in June and one in July.

What was the plague history of these 37 villages in the following epidemics of which that of 1905—06 was slight and that of 1906—07 was severe? Eight were not infected in either of these epidemics, 20 were infected in 1906—07 but not in 1905—06, leaving only nine which returned deaths in the latter epidemic. In two of these nine villages very few deaths occurred, six or under, which suggests that there may have been no indigenous plague in them.

When we now consider the interval of freedom from deaths which the 29 villages subsequently infected enjoyed we find (Table XLII) that the great majority of them were free for a long period, 19—23 months, and that all the nine villages which returned deaths in the epidemic of 1905—06 were free for at least five months and the majority of them for 8—9 months.

(*c*) *Villages infected at the end of the epidemic of* 1905—06. Of the villages infected during this mild epidemic only four still reported deaths in June, so that we shall include in our analysis eight others in which the last deaths occurred in May, 12 villages in all. Of these 12 villages three returned no deaths while nine became infected in the epidemic of 1906—07, which it will be remembered was severe and widespread. When we analyse (Table XLII) the data referring to the interval during which these villages returned no plague deaths it is seen that two had a free interval of 2 to 3 months, while the remaining seven villages did not report deaths till late on in the epidemic, when many other villages were already infected. They had enjoyed a free interval of from 7 to 10 months.

B. *Mozuffarnagar District.*

(*a*) *Villages infected at the end of epidemic of* 1902—03. In the epidemic of 1902—03, 11 villages still returned deaths in May or June 1903. Of these one was not again infected, while the period of freedom enjoyed by the other ten varied from 3 to 20 months. From Table

XLIII it will be seen that the great majority did not again report deaths until after an interval of more than eight months.

(*b*) *Villages infected at the end of epidemic of* 1903—04. In the epidemic of 1903—04, 22 villages were still returning plague deaths in June 1904. Two of these had no free interval, deaths continuing to occur right through the off season; two others had an interval of freedom of one and two months respectively; further, 10 more villages returned deaths in the epidemic of 1904—05, but did not begin until late on in the epidemic, at a time when many other villages were infected. The remaining eight villages were not infected till a subsequent epidemic, escaping altogether during the 1904—05 outbreak.

(*c*) *Villages infected at the end of epidemic of* 1904—05. In the epidemic of 1904—05, 35 villages returned plague deaths as late as June 1905. Of these villages seven were not again infected, 23 did not return deaths till the epidemic of 1906—07, while the remaining five were infected in the epidemic of 1905—06 but not until late on in the course of the outbreak.

(*d*) *Villages infected at the end of epidemic of* 1905—06. In the epidemic of 1905—06, which it will be remembered was a very mild one, only six villages reported deaths in June. All these were again infected during the severe epidemic of 1906—07, but deaths did not begin to occur till the epidemic was fairly well advanced, the interval of freedom enjoyed being from 4 to 8 months.

C. *Amritsar District.*

It is unnecessary to do anything further than draw attention to the table containing the data (XLI) and that showing the analysis (XLIV). These are of the same nature as those already described for Rohtak and Mozuffarnagar districts.

D. *Summary.*

Villages which return deaths at the end of one epidemic may or may not show infection during the next epidemic. Only in a very few instances is there no interval free from plague deaths between one epidemic and another and in a few more the interval is from 1 to 4 months. On the other hand the great majority of the villages infected at the end of an epidemic do not report deaths early in the subsequent epidemic. They are either not infected at all or the free interval is from 6 to 10 months. By this time the epidemic is already widespread and many other villages are infected.

VII. THE QUESTION WHETHER PLAGUE TENDS TO RECUR IN VILLAGES IN SUCCESSIVE EPIDEMICS.

As this question is elsewhere fully discussed by Dr Greenwood we need not do more than refer to the tables annexed and to the general conclusions these would appear to us to support.

We would remind our readers that in our study of plague in the Punjab villages of Dhand and Kasel (*Journal of Hygiene*, vol. VII. p. 984) an attempt was made to determine whether houses which were infected in one epidemic were especially liable to be again infected in any subsequent epidemic, and that we arrived at the very definite conclusion that plague showed no tendency to recur in houses during successive epidemics. The same method was now used to ascertain if the villages in the three districts of Rohtak, Mozuffarnagar and Amritsar owed their infection to chance or not.

The data are given for Rohtak in Tables XLV to XLVII, for Mozuffarnagar in Tables XLVIII to L, and for Amritsar in Tables LI to LIII.

From a study of these tables which show a marked lack of correspondence between the actual and calculated figures it is evident that chances of infection were not altogether random, that is to say, that some villages were more liable to be infected than others.

On thinking over the problem it suggested itself to us that the population of the villages might be an important factor in determining whether a village would become infected or not. We have roughly tested the truth of this hypothesis in all three districts, first by comparing the average population of the villages infected in each of the epidemics, it being remembered that the epidemics varied greatly in severity, and secondly by comparing the average population of the villages infected in none of the epidemics, in one epidemic only, in any two, three etc. epidemics.

These data are contained in Tables LIV to LV for Rohtak district, Tables LVI to LVII for Mozuffarnagar district and Tables LVIII to LIX for Amritsar district. These tables all show the same phenomena.

First, it is seen that in the epidemics in which only a small number of villages returned deaths the average population of these villages was very much greater than that of the villages infected in the years in which a large number reported deaths, that in fact, there was an inverse proportion between the number of villages attacked in an epidemic and the average population of these villages.

Secondly, it is seen that the average population of the villages which have enjoyed complete immunity is very small and that the greater the number of times the villages are infected the greater the average population. There is in fact a direct proportion between the number of epidemics in which villages are infected and the average population of the villages.

Thirdly, in the case of Rohtak there is another piece of evidence which shows that population is an important factor in determining the chances of infection of a village. In an early part of this paper we draw attention to the fact that in the Jhaggar tehsil of Rohtak district there were not only a smaller number of inhabitants but also a larger number of villages than in any of the other three tehsils, the result being that the average population of the villages in this subdivision was comparatively small, much below that of the other tehsils and of the district as a whole. This being so we should expect to find that the villages of the Jhaggar tehsil had suffered less than those of the other tehsils. And this expectation is shown to be correct both from the figures set forth in Table LX and from the maps 1 to 6.

From the table it is seen that, while 51·9 p.c. of the villages in Jhaggar tehsil were never infected with plague, in the other tehsils this percentage was between 7 and 19. Further it is seen that the percentage of villages in Jhaggar infected in two and three epidemics was very much less than in Rohtak, Gohana and Sampla.

In the maps 1 to 5 the villages infected in none of the epidemics, in only one epidemic, in any two, in any three and in all four epidemics respectively have been marked out. It is seen at once that as regards villages infected in all four epidemics there is no marked grouping, that Jhaggar is remarkably free from villages infected in any three epidemics and contains much the greatest number of villages infected in two epidemics and those never infected at all.

SUMMARY.

By comparing the actual number of villages infected in none of the epidemics, in only one, in any two etc. epidemics with the number calculated on the assumption that all villages are equally liable to become infected, it is evident from the lack of correspondence between the actual and calculated figures that the assumption is not correct, that, in fact, some villages are more liable to become infected than others.

On further investigation it would appear that one factor at least which determines this greater liability to infection is the number of inhabitants, the larger villages being more often infected than the smaller.

TABLE I.

Showing the distribution of the population in the four Tehsils of the Rohtak District.

Tehsil	No. of villages	Population	Average population per village
Gohana	79	140,682	1781
Rohtak	109	195,423	1793
Sampla	124	160,262	1292
Jhaggar	187	124,455	665

TABLE II.

Showing a summary of the plague history of Rohtak District for each epidemic:

Epidemic of	Number of villages which returned deaths	Total mortality about
1903—04	37	2500
1904—05	285	27,000
1905—06	30	2000
1906—07	249	30,000

TABLE III.

Showing the number of villages infected month by month in each Tehsil of the Rohtak District.

Month	Year	Rohtak	Gohana	Sampla	Jhaggar	Total
January	1904	3	0	0	1	4
	1905	26	7	14	16	63
	1906	2	3	1	0	6
	1907	4	11	6	0	21
February	1904	9	0	1	5	15
	1905	36	9	34	22	101
	1906	3	5	1	0	9
	1907	10	25	13	1	49
March	1904	15	0	5	11	31
	1905	51	18	51	36	156
	1906	3	8	0	0	11
	1907	33	41	52	6	132
April	1904	15	1	8	13	37
	1905	65	46	68	53	232
	1906	8	11	1	2	22
	1907	52	57	68	19	196
May	1904	15	1	6	13	35
	1905	55	50	65	38	208
	1906	8	11	0	1	20
	1907	55	55	70	19	199
June	1904	7	1	2	2	12
	1905	7	22	33	7	69
	1906	0	4	0	0	4
	1907	34	19	44	13	110
July	1904	1	0	0	0	1
	1905	0	0	1	0	1
	1906	0	0	0	0	0
	1907	3	3	3	0	9
August	1904	0	0	1	1	2
	1905	0	0	0	0	0
	1906	1	0	0	0	1
	1907	1	0	0	0	1
September	1904	0	1	3	2	6
	1905	0	0	0	0	0
	1906	1	2	1	0	4
October	1904	2	1	3	3	9
	1905	0	1	1	0	2
	1906	1	4	2	0	7
November	1903	3	0	0	0	3
	1904	5	1	3	8	17
	1905	0	1	1	0	2
	1906	1	6	2	0	9
December	1903	4	0	0	0	4
	1904	7	4	5	7	23
	1905	0	2	1	0	3
	1906	1	8	2	0	11

TABLE IV

Showing the data referring to the five epidemics in Mozuffarnagar District.

Number and year of epidemic		Number of villages which returned deaths	Total mortality about
1st	1902—3	25	1256
2nd	1903—4	130	8777
3rd	1904—5	313	11,867
4th	1905—6	69	2962
5th	1906—7	579	34,933

TABLE V.

Showing month by month the number of villages which reported plague deaths in Mozuffarnagar District.

Year	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
1902	1	0	0	1	0	0	0	0	0	0	5	2
1903	2	5	9	15	11	3	0	0	1	2	5	7
1904	11	30	51	91	52	22	4	7	12	19	36	64
1905	83	100	163	173	150	35	0	1	1	1	5	7
1906	7	12	29	46	33	6	1	1	1	7	16	35
1907	77	159	288	438	427	169	7	2	2	5	6	10

TABLE VI.

Showing data referring to the six epidemics in the Amritsar District.

Number and year of epidemic		Number of villages which returned deaths	Total mortality about
1st	1901—02	62	2509
2nd	1902—03	506	26,181
3rd	1903—04	445	22,437
4th	1904—05	669	29,930
5th	1905—06	276	8535
6th	1906—07	604	24,503

TABLE VII.

Showing month by month the number of villages which reported plague deaths in Amritsar District.

Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1902	0	2	8	23	30	24	7	1	1	4	46	82
1903	118	180	153	299	214	189	32	1	0	0	2	2
1904	24	47	116	329	410	117	14	1	4	17	37	93
1905	178	285	389	461	456	245	39	1	1	1	4	4
1906	14	22	62	158	222	75	11	1	2	5	20	39
1907	59	118	211	411	480	354	69	—	—	—	—	—

TABLE VIII. (*Rohtak District.*)

Showing analysis of data referring to previous plague history of villages infected each epidemic.

Number and year of epidemic		No. of villages infected	No. and percentage of villages never before infected	No. and percentage of villages infected in previous epidemic	No. and percentage of villages which escaped infection for one epidemic	No. and percentage of villages which escaped infection for two epidemics
1st	1903—04	57	57 (100 p.c.)	—	—	—
2nd	1904—05	285	241 (85 p.c.)	44 (15 p.c.)	—	—
3rd	1905—06	30	4 (13·3 p.c.)	26 (86·7 p.c.)	—	—
4th	1906—07	249	52 (20·9 p.c.)	26 (10·4 p.c.)	164 (65·9 p.c.)	7 (2·8 p.c.)

TABLE IX. (*Mozuffarnagar District.*)

Showing analysis of data referring to the previous plague history of villages infected each epidemic.

Number and year of epidemic		No. of villages infected	No. and percentage of villages never before infected	No. and percentage of villages infected in previous epidemic	No. and percentage of villages which escaped infection during one epidemic	No. and percentage of villages which escaped infection in two epidemics	No. and percentage of villages which escaped infection in three epidemics
1st	1902—03	25	25 (100 p.c.)	—	—	—	—
2nd	1903—04	130	121 (93 p.c.)	9 (7 p.c.)	—	—	—
3rd	1904—05	313	220 (70·3 p.c.)	82 (26·2 p.c.)	11 (3 p.c.)	—	—
4th	1905—06	69	24 (34·8 p.c.)	41 (59·4 p.c.)	4 (5·8 p.c.)	—	—
5th	1906—07	579	249 (43 p.c.)	62 (10·7 p.c.)	227 (39·4 p.c.)	39 (6·6 p.c.)	2 (0·3 p.c.)

TABLE X. (*Amritsar District.*)

Showing analysis of data referring to the previous plague history of villages infected each epidemic.

Number and year of epidemic		Total no. of villages infected	No. and percentage of villages never before infected	No. and percentage of villages infected in previous epidemic	No. and percentage of villages which escaped infection in one epidemic only	No. and percentage of villages which escaped infection in two epidemics	No. and percentage of villages which escaped infection in three epidemics	No. and percentage of villages which escaped infection in four epidemics
1st	1901—02	62	62 (100 p.c.)	—	—	—	—	—
2nd	1902—03	506	462 (91·3 p.c.)	44 (8·7 p.c.)	—	—	—	—
3rd	1903—04	445	149 (33·5 p.c.)	286 (64·2 p.c.)	10 (2·3 p.c.)	—	—	—
4th	1904—05	669	166 (24·8 p.c.)	345 (51·6 p.c.)	153 (22·9 p.c.)	5 (0·7 p.c.)	—	—
5th	1905—06	276	9 (3·3 p.c.)	230 (83·3 p.c.)	26 (9·5 p.c.)	10 (3·6 p.c.)	1 (0·3 p.c.)	—
6th	1906—07	604	59 (9·8 p.c.)	201 (33·3 p.c.)	290 (48 p.c.)	31 (5·1 p.c.)	22 (3·6 p.c.)	1 (0·2 p.c.)

TABLE XI.

Data referring to villages in Rohtak District infected in the beginning of the 1903—04 *epidemic.*

No. of village	Tehsil	Population	Month when first infected	Remarks
112	Rohtak	9723	November '03	—
28	,,	7824	,,	—
32	,,	4191	,,	—
113	,,	609	January '04	Probably infected from 112 in immediate neighbourhood.
114	,,	519	,,	
194	Jhaggar	2469	,,	—
171	,,	3896	February '04	—
135	,,	3602	,,	—
62	,,	12,227	,,	—
81	Sampla	598	,,	Infected probably from Beri neighbourhood.

N.B. As this was the first epidemic none of these villages had been previously infected.

TABLE XII.

Data referring to villages in Rohtak District infected at the beginning of the 1904—05 *epidemic.*

No. of village	Tehsil	Population	Month when first infected in 1904-05 epidemic	Month when last death was reported in 1903-04 epidemic	No. of months free from deaths	Remarks
81	Sampla	598	August	May	2	Infected Feb.—May 04.
164	Jhaggar	531	,,	Nil	—	—
62	,,	12,227	September	May	3	Infected Feb.—May 04.
93	Sampla	5316	,,	June	2	Infected Mar.—June 04.
74	,,	5060	,,	June	2	Infected Mar.—June 04.
26	Gohana	2343	,,	Nil	—	—
85	Rohtak	20,024	October	May	4	Had a few deaths Feb. —May 04.
112	,,	9723	,,	June	3	Infected Feb.—May 04.
32	Jhaggar	1104	,,	May	4	Had a few deaths Ap.—May 04.
76	Sampla	286	,,	Nil	—	—
53	Rohtak	5024	November	May	5	Few deaths Ap.—May 04.
40	,,	1931	,,	Nil	—	—
90	,,	1173	,,	Nil	—	—
135	Jhaggar	3602	,,	April	6	Few deaths Feb.—Ap. 04.
102	,,	885	,,	Nil	—	—
94	Rohtak	1735	December	Nil	—	—
74	Jhaggar	1823	,,	Nil	—	—
20	Sampla	3309	,,	Nil	—	—
16	Gohana	7509	,,	Nil	—	—
56	,,	485	,,	Nil	—	—
59 A	,,	383	,,	Nil	—	—

TABLE XIII.

Data referring to villages in Rohtak District infected at the beginning of the 1905—06 *epidemic.*

No. village	Tehsil	Population	Month when first infected in 1905-06 epidemic	Month when last death was reported	No. of months free from deaths	Remarks
31	Gohana	4241	October	June 05	3	—
104	Sampla	266	,,	May 05	4	Only village infected in Sampla Tehsil in this epidemic.
3	Gohana	2443	December	June 05	5	—
13	,,	3185	January	June 05	6	—
21	Rohtak	2783	,,	May 05	7	—
112	,,	9723	,,	May 05	7	—

TABLE XIV.

Data referring to villages in Rohtak District infected at beginning of the 1906—07 *epidemic.*

No. of village	Tehsil	Population	Month when first infected in 1906-07 epidemic	Month when last death was reported	No. of months free from deaths
11	Rohtak	5126	August	May 06	2
17	Gohana	5836	September	May 05	15
31	,,	4241	,,	June 06	2
12	Sampla	3765	,,	May 05	15
32	,,	2606	October	June 05	15
39	Gohana	5657	,,	June 06	3
58	,,	1660	,,	May 05	16
47	,,	2470	November	May 06	5
26	,,	2343	,,	Jan. 05	21
27	,,	3247	December	May 05	18
57	,,	1178	,,	May 05	18

TABLE XV.

Data referring to villages in Rohtak District in which the infection was probably carried over each year from one epidemic to the other as acute rat plague.

No. of village	Tehsil	Population	Months when first and last deaths took place in 1st epidemic	No. of deaths in 1st epidemic	Month when 1st death took place in 2nd epidemic	No. of months free from deaths
Between 1st and 2nd epidemics:						
81	Sampla	698	Feb.—May 04	61	August 04	2
93	,,	5316	Mar.—June 04	100	Sept. 04	2
74	,,	5060	Ap.—June 04	75	Sept. 04	2
62	Jhaggar	12,227	Feb.—May 04	42	Sept. 04	3
112	Rohtak	9723	Feb.—June 04	519	Oct. 04	3
Between 2nd and 3rd epidemics:						
31	Gohana	4241	May—June 05	31	Oct. 05	3
104	Sampla	266	Ap.—May 05	5	Oct. 05	4
Between 3rd and 4th epidemics:						
11	Rohtak	5126	Ap.—May 06	13	Aug. 06	2
31	Gohana	4241	May—June 06	55	Sept. 06	2
39	,,	5657	Ap.—June 06	32	Oct. 06	3

TABLE XVI.

Data referring to villages in Mozuffarnagar District infected at the beginning of the epidemic of 1903—04.

Name	Population	Thana	Month when infection began	Month when last previous death occurred	No. of months free	Remarks
Kadargarh	618	Thana Bawan	Sept. 03	Nil	—	—
Mozuffarnagar	23,444	Mozuffarnagar	Oct. 03	June 03	3	—
Khatauli	8695	Khatauli	,,	,,	3	—
Sandholi	418	,,	Nov. 03	Nil	—	—
Mukimpur	2668	Meranpur	,,	Nil	—	—
Kailaoda Kalan	2140	Khatauli	,,	May 03	5	Only 1 case in ep. of 1902–03.
Naola	3752	,,	Dec. 03	Nil	—	
Titawa	1192	,,	,,	Nil	—	—
Jauli	2579	Bhopa	,,	Nil	—	—
Khandla	11,563	Khandla	,,	Nil	—	—
Sarai	2752	Khatauli	Jan. 04	Nil	—	—
Barsu	1457	,,	,,	Nil	—	—
Pipalhera	1184	,,	,,	Nil	—	—
Nagli	542	,,	,,	Nil	—	—
Jansath	6507	Jansath	,,	Nil	—	—
Kukra	3205	Shahpur	,,	Nil	—	—

TABLE XVII.

Data referring to villages in Mozuffarnagar District infected at the beginning of the epidemic of 1904—05.

Name	Population	Thana	Month when infection began	Month when last previous death occurred	No. of months free	Remarks
Tijalhera	2385	Purkazi	July 04	June 04	0	—
Belra	1809	Bhopa	,,	,,	0	—
Gianna Mazra	790	Charthawal	,,	Nil	—	—
Arnaki	167	,,	,,	Nil	—	—
Meranpur	7209	Meranpur	Aug. 04	June 04	1	Only 1 death in Aug. 04.
Jandheri	1019	Jansath	,,	Nil	—	
Goela	3098	Shahpur	,,	Nil	—	—
Harsauli	3069	Titawi	,,	Nil	—	—
Rani	1753	Charthawal	,,	Nil	—	—
Pur	6384	Purkazi	Sept. 04	May 04	3	Only 2 deaths in Sept. 04.
Tisa	3384	Bhopa	,,	June 04	2	
Chaurawala	1543	,,	,,	Nil	—	—
Kethora	2668	Meranpur	,,	April 04	4	Only 2 deaths in April 04.
Chandam	471	Jansath	,,	Nil	—	
Kalyanpur	830	Shahpur	,,	Nil	—	—
Bitanda	2663	Budhana	,,	April 03	17	—
Bhukarheri	6316	Bhopa	Oct. 04	Nil	—	—
Murahlpur	3	,,	,,	Nil	—	—
Barkara	1220	,,	,,	Nil	—	—
Karehra	1220	,,	,,	Nil	—	—
Kanarhen	527	Charthawal	,,	Nil	—	—
Rasulpur Khurd	418	Meranpur	,,	April 04	5	Only 1 death in April 04.
Antwara	1721	Jansath	,,	,,	5	Ditto.
Basayach	889	,,	,,	Nil	—	—
Karthal	1823	Budhana	,,	May 03	17	—
Mandoli	313	,,	,,	April 03	18	—

TABLE XVIII.

Data referring to villages in Mozuffarnagar District infected at the beginning of the epidemic of 1905—06.

Name	Population	Thana	Month when infection began	Month when last previous death occurred	No. of months free	Remarks
Jansath	6507	Jansath	Aug. 05	May 05	2	—
Loi	7095	Kandhla	Oct. 05	Nil	—	—
Kutesra	3565	Charthawal	Nov. 05	Mar. 05	7	—
Kamhera	1197	Jansath	,,	,,	—	Only 1 death in 1904—05.
Balla Mazra	771	Chausana	,,	April 05	—	Only 2 deaths in 1904—05.
Bhuma	1893	Meranpur	,,	May 05	—	Only 1 death in 1905—06.
Dathera	1245	Chausana	Dec. 05	April 05	—	Ditto.
Shamli	7478	Shamli	,,	June 05	5	—
Karnali	1078	,,	,,	Nil	—	—
Mustgarh	359	Thanabhawan	Jan. 06	Nil	—	—
Harsauli	3069	Titawi	,,	May 05	—	Only 1 death in 1904—05.
Balwa	2503	Shamli	Feb. 06	Mar. 05	—	Ditto.
Lank	3863	,,	,,	June 04	19	—
Mandwara	607	Budhana	,,	Nil	—	—
Nirmani	877	Titawi	,,	Nil	—	—
Ghesu Khera	571	Charthawal	,,	Jan. 05	—	Only 1 death in 1905—06.
Pura	805	Khatauli	,,	Nil	—	
Gadla	1767	Bhopa	,,	Jan. 05	12	—
Bhu Karheri	6316	,,	,,	Feb. 05	11	—

TABLE XIX.

Data referring to villages in Mozuffarnagar District infected at the beginning of the epidemic of 1906—07.

Name	Population	Thana	Month when infection began	Month when last previous death occurred	No. of months free	Remarks
Kandla	11,563	Kandla	July 06	May 06	1	1 death in July. 1 death in Aug. 7 deaths in Nov.
Kasauli	1323	Charthawal	Sept. 06	,,	—	Only 1 death in 1906—07.
Rasulpur	851	Shahpur	Oct. 06	April 04	29	—
Nizampur	299	Khatauli	,,	Nil	29	—
Meranpur	7209	Meranpur	,,	April 04	17	—
Wazirabad	669	Bhopa	,,	Feb. 05	19	—
Chachrauli	871	,,	,,	Nov. 04	22	—
Yusafpur	824	,,	,,	Nil	22	—
Chaurawala	1543	,,	,,	May 06	4	—
Alayarpur	552	Shahpur	Nov. 06	Nil	4	—
Baghrli	4935	Titawi	,,	May 05	17	—
M. Nagar	23,444	M. Nagar	,,	June 06	4	—
Chandpur	1109	,,	,,	May 06	5	—
Bilaspur	1390	,,	,,	Feb. 05	20	—
Khojahera	999	Jansath	,,	May 05	17	—
Sikri	3026	Bhopa	,,	May 06	5	—
Jauli	2579	,,	,,	June 06	4	—
Pur	6384	Purkazi	,,	May 05	17	—
Garhi-Hasanpur	1461	Chausana	,,	May 05	17	—
Toda	800	,,	Dec. 06	Nil	—	—
Bhikki-Mazra	487	Shamli	,,	May 05	17	—
Amernagar	1939	Titawi	,,	Mar. 05	20	—
Kanami	2508	,,	,,	May 05	18	—
Makhyali	1851	M. Nagar	,,	Feb. 05	21	—
Dhudhera	872	,,	,,	Nil	—	—
Khatauli	3695	Khatauli	,,	Mar. 05	20	—
Talra	1214	Jansath	,,	May 06	—	Only 1 death, May 06.
Palri	520	,,	,,	May 05	18	
Mahalki	1365	,,	,,	April 05	19	—
Antnara	1721	,,	,,	,,	19	—
Jansath	6507	,,	,,	June 06	5	—
Chitaura	1762	,,	,,	May 06	6	—
Karandah	1349	,,	,,	Nil	—	—
Kethora	2668	Meranpur	,,	Mar. 05	18	—
Gadhi-Rasulpur	418	,,	,,	Nil	—	—
Mukimpur	2668	,,	,,	Feb. 05	21	—
Kakranli	3985	Bhopa	,,	,,	21	—
Teora	2699	,,	,,	June 06	—	Only 1 death, May 05—06.
Berah-Sadat	1522	,,	,,	Feb. 05	21	
Bhoapur	676	,,	,,	Nil	—	—
Malpura	635	,,	,,	Nil	—	—
Khudda	2441	Purkazi	,,	May 04	30	—
Harainti	519	,,	,,	Nil	—	—
Lakhnanti	300	,,	,,	Nil	—	—
Aterna	1304	Budhana	,,	Nil	—	—

TABLE XX.

Data referring to villages in Mozuffarnagar District in which the infection was probably carried over from one epidemic to another as acute rat plague.

Name	Thana	Population	Months when first and last deaths took place in 1st epidemic	No. of deaths in 1st epidemic	Month when first death took place in 2nd epidemic	No. of months free from deaths
Mozuffarnagar	M. Nagar	23,444	Feb.—June 03	25	Oct. 03	3
Khatauli	Khatauli	8695	Mar.—June 03	4	Oct. 03	3
Tijalhera	Purkazi	2385	April—July 04	120	Aug. 04	0
Belra	Bhopa	1809	May—July 04	68	Aug. 04	0
Meranpur	Meranpur	7209	Mar.—June 04	151	Aug. 04	1
Pur	Purkazi	6384	Mar.—May 04	279	Sept. 04	3
Tisa	Bhopa	3384	April—June 04	29	Sept. 04	2
Jansath	Jansath	6507	Jan.—May 05	146	Aug. 05	2
Kandla	Kandla	11,563	April—May 06	52	July 06	1

TABLE XXI.

Data referring to villages in Amritsar District infected at beginning of 1902—03 *epidemic.*

No.	Tehsil	Population	Month when became infected	Month of last previous death	No. of months free	Remarks
113	Amritsar	162,429	Aug. 02	July 02	0	Amritsar City
24	,,	1525	Oct. 02	June 02	3	3 deaths, May —June 02.
17	,,	6490	,,	Nil	—	
144	Ajnala	429	,,	Nil	—	—
110	Amritsar	990	Nov. 02	Nil	—	—
38	,,	317	,,	Nil	—	—
202	,,	230	,,	Nil	—	—
175	,,	238	,,	Nil	—	—
42	,,	407	,,	Nil	—	—
222	,,	868	,,	Nil	—	—
60	,,	946	,,	Nil	—	—
138	,,	307	,,	Nil	—	—
16	,,	958	,,	Nil	—	—
143	,,	1470	,,	July 02	3	—
63	,,	1700	,,	,,	3	1 death in Nov. 02.
109	,,	1323	,,	Nil	—	
39	,,	1090	,,	Nil	—	1 death in July 02.
171	,,	1144	,,	June 02	4	—
201	,,	1966	,,	Nil	—	—
239	,,	1076	,,	Nil	—	—
67	,,	1019	,,	Nil	—	—
176	,,	1863	,,	Nil	—	—
64	,,	1117	,,	Nil	—	—
65	,,	1826	,,	Nil	—	—
169	,,	1959	,,	Nil	—	—
27	,,	5029	,,	Nil	—	—
64	,,	1130	,,	Nil	—	—
46	Tarn-Tarn	1114	,,	June 02	4	—
28	,,	1628	,,	Nil	—	—
42	,,	2208	,,	June 02	4	—
49	,,	2440	,,	July 02	3	—
80	,,	1742	,,	Nil	—	—
41	,,	4161	,,	Nil	—	—
165	,,	331	,,	Nil	—	—
121	,,	1067	,,	Nil	—	—
150	,,	3206	,,	Nil	—	—
100	,,	930	,,	Nil	—	—
33	,,	518	,,	Nil	—	—
120	,,	4428	,,	Nil	—	—
146	Ajnala	564	,,	Nil	—	—
270	,,	2439	,,	Nil	—	—
145	,,	1514	,,	Nil	—	—
162	,,	1166	,,	Nil	—	—
180	,,	405	,,	Nil	—	—
161	,,	954	,,	Nil	—	—
162 A	,,	349	,,	Nil	—	—

TABLE XXII.

Data referring to villages in Amritsar District infected at the beginning of 1903—04 *epidemic.*

No.	Tehsil	Population	Month when became infected	Month of last previous death	No. of months free	Remarks
713	Amritsar	162,429	Aug. 03	July 03	0	—
63	,,	1700	Nov. 03	Nov. 02	11	1 case in Nov. 02.
258	Ajnala	2179	,,	April 03	6	—
57	Amritsar	1500	Jan. 04	June 03	6	—
143	,,	1470	,,	Jan. 03	11	—
83	,,	878	,,	June 03	6	—
40	,,	1868	,,	May 03	7	—
196	,,	1600	,,	June 02	18	—
262	,,	3029	,,	May 03	7	—
136	,,	258	,,	Nil	—	—
146	,,	5817	,,	June 03	6	—
236	,,	916	,,	April 03	8	—
268	,,	1158	,,	May 03	7	—
216	,,	2494	,,	June 03	6	—
89	,,	3709	,,	April 03	8	—
176	,,	1863	,,	Feb. 03	10	—
138	Tarn-Tarn	801	,,	May 03	7	—
264	,,	4343	,,	June 03	6	—
323	,,	1846	,,	Nil	—	—
41	,,	4161	,,	Feb. 03	10	—
67	,,	1597	,,	Mar. 03	9	—
120	,,	4428	,,	April 03	8	—
311	,,	3654	,,	Nil	—	—

TABLE XXIII.

Data referring to villages in Amritsar District infected at the beginning of 1904—05 *epidemic.*

No.	Tehsil	Population	Month when became infected	Month of last previous death	No. of months free	Remarks
1	Amritsar	263	Aug. 04	June 03	13	2 deaths only in Aug., 1 death in Sept.
167	,,	715	Sept. 04	Nil	—	
25	,,	637	,,	Nil	—	
261	,,	1033	,,	May 03	15	—
189	Tarn-Tarn	730	,,	Nil	—	—
57	Amritsar	1500	Oct. 04	May 04	4	—
306	,,	524	,,	Nil	—	—
292	,,	1776	,,	Nil	—	—
103	,,	867	,,	April 04	5	—
102	,,	209	,,	Nil	—	—
317	,,	1062	,,	June 04	3	—
308	,,	843	,,	Feb. 03	19	—
291	,,	430	,,	Nil	—	—
142	Tarn-Tarn	2107	,,	June 04	3	—
241	,,	792	,,	June 03	15	—
243	,,	3538	,,	May 03	16	—
31	,,	238	,,	May 04	4	—
159	,,	1513	,,	June 04	3	—
210	Ajnala	1891	,,	July 03	14	—
146	,,	1514	,,	May 04	4	—
100	Amritsar	1517	Nov. 04	June 03	16	—
163	,,	1411	,,	June 03	16	—
327	,,	1143	,,	Nil	—	—
267	,,	1011	,,	June 03	16	—
116	,,	639	,,	Nil	—	—
78	,,	1470	,,	May 04	5	—
77	,,	611	,,	June 03	16	—
288	,,	1073	,,	June 04	4	—
276	,,	1772	,,	June 04	4	—
343	,,	1062	,,	June 04	4	—
32	,,	502	,,	Nil	—	—
176	,,	1863	,,	April 04	6	—
51	Tarn-Tarn	326	,,	June 03	16	—
190	,,	220	,,	May 03	17	—
269	,,	3400	,,	May 04	5	—
230	,,	247	,,	June 03	16	—
66	,,	1090	,,	June 04	4	—
226	,,	3291	,,	June 03	16	—
67	,,	1597	,,	May 04	5	—
215	,,	2737	,,	June 03	16	—
85	,,	1201	,,	May 04	5	2 deaths in May 04.
273	,,	581	,,	Nil	—	

TABLE XXIV.

Data referring to villages in Amritsar District infected at the beginning of 1905—06 *epidemic.*

No.	Tehsil	Population	Month when became infected	Month of last previous death	No. of months free	Remarks
113	Amritsar	162,429	Aug. 05	July 05	0	—
700	,,	1517	Nov. 05	Mar. 05	7	—
60	,,	946	,,	May 04	17	—
59	,,	154	,,	May 04	17	1 death in May 04.
175	,,	238	Jan. 06	April 05	8	—
71	,,	1794	,,	April 05	8	—
249	,,	1306	,,	May 05	7	—
159	,,	533	,,	Mar. 05	9	—
72	,,	1520	,,	April 05	8	—
86	,,	1829	,,	June 05	6	—
78	,,	1470	,,	Mar. 05	9	—
57	Tarn-Tarn	1177	,,	Feb. 05	10	—
66	,,	1090	,,	July 05	5	—
69	,,	469	,,	May 04	19	—
113	,,	2463	,,	May 05	7	1 death in Jan. 06.
59	,,	1433	,,	July 05	5	—
96	Amritsar	1602	Feb. 06	June 05	6	—
275	,,	2431	,,	Mar. 05	9	—
201	,,	432	,,	May 05	7	—
261	,,	1033	,,	Mar. 05	9	—
111	,,	1364	,,	June 05	6	—
288	,,	1520	,,	April 05	8	—
37	,,	2830	,,	June 04	18	—
42	Tarn-Tarn	2208	,,	June 04	18	—
287	Ajnala	763	,,	June 05	6	—
109	,,	692	,,	April 05	8	1 death in Feb. 06.

TABLE XXV.

Data referring to villages in Amritsar District infected at the beginning of 1906—07 *epidemic.*

No.	Tehsil	Population	Month when became infected	Month of last previous death	No. of months free	Remarks
113	Amritsar	162,429	Aug. 06	July 06	0	Last death 25. 8. 06, then in Jan. 07.
291	,,	430	Sept. 06	Dec. 04	20	
235	Tarn-Tarn	979	,,	June 06	2	
123	,,	2303	Oct. 06	June 05	15	—
183	Ajnala	843	,,	May 03	40	—
165	,,	323	,,	Nil	—	—
43	Amritsar	1479	Nov. 06	May 05	17	—
57	,,	1500	Nov. 04	Dec. 04	22	—
322	,,	1335	,,	Feb. 05	20	—
69	,,	993	,,	June 05	16	—
302	,,	734	,,	May 06	5	—
38	,,	511	,,	April 04	30	—
78	,,	1470	,,	April 06	6	—
72	Tarn-Tarn	1298	,,	April 05	18	—
147	Ajnala	773	,,	Nil	—	—
5	,,	1209	,,	May 04	29	1 death in 1906—07.
181	,,	943	,,	May 05	17	
213	,,	775	,,	May 03	41	—
113	,,	957	,,	May 05	17	—
245	,,	3198	,,	June 05	16	—
183 A	,,	843	,,	May 03	41	—
124	Amritsar	436	Dec. 06	April 05	19	—
325	,,	2090	,,	June 06	5	—
312	,,	2110	,,	May 06	6	1 death in 1906—07.
284	,,	692	,,	April 05	19	
96	,,	1602	,,	May 06	6	—
279	,,	1664	,,	July 06	4	—
143	,,	1470	,,	May 05	18	—
240	,,	234	,,	May 05	18	—
229	,,	1779	,,	June 06	5	—
318	,,	897	,,	May 05	18	2 deaths in Dec. no more till April.
123	,,	321	,,	Nil	—	
101	,,	1346	,,	June 06	5	
41	,,	319	,,	May 06	6	—
341	,,	1119	,,	June 06	5	—
26	,,	5029	,,	May 05	18	—
206	Tarn-Tarn	951	,,	Feb. 05	21	—
206 A	,,	1467	,,	June 06	5	—
21	Ajnala	374	,,	May 05	18	2 deaths only in 1906—07.
162	,,	1166	,,	May 05	18	
161	,,	383	,,	Nil	—	—
273	,,	4511	,,	June 06	5	—
245 A	,,	3198	,,	June 04	29	—
181 A	,,	298	,,	May 05	18	—

TABLE XXVI.

Data referring to villages in Amritsar District in which the infection was probably carried over from one epidemic to another as acute rat plague.

No.	Tehsil	Population	Months when first and last deaths took place in 1st epidemic	No. of deaths in 1st epidemic	Month when first death took place in 2nd epidemic	No. of months free from deaths
113	Amritsar	162,429	Feb.—July 02	82	Aug. 02	0
24	,,	1525	May—June 02	3	Oct. 02	3
143	,,	1470	April—July 02	35	Nov. 02	3
63	,,	1700	July 02	2	Nov. 02	3
49	Tarn-Tarn	2440	May—July 02	14	Dec. 02	3
113	Amritsar	162,429	Aug. 02, July 03	460	Aug. 03	0
317	,,	1062	May—June 04	3	Oct. 04	3
142	Tarn-Tarn	2107	April—June 04	25	Oct. 04	3
159	,,	1513	April—June 04	13	Oct. 04	3
113	Amritsar	162,429	Jan.—July 05	1073	Aug. 05	0
113	,,	162,429	Aug. 05, July 06	1903	Aug. 06	0
235	Tarn-Tarn	979	May—June 06	15	Sept. 06	2

TABLE XXVII.

Data referring to villages in Rohtak District infected in the epidemic of 1903—04.

No. of village	Tehsil	Population	Details of infection of 1903—04		Details of next subsequent infection		No. of months free from deaths
			Months during which deaths were returned	No. of deaths	Months during which deaths were returned	No. of deaths	
100	Rohtak	1032	Feb.—May	31	Nil	Nil	—
186	Jhaggar	210	May	1	Nil	Nil	—
71	,,	384	April—May	8	Nil	Nil	—
109	,,	361	Mar.—May	8	Nil	Nil	—
78	,,	767	Mar.—May	54	Nil	Nil	—
14	,,	419	May	1	Nil	Nil	—
95	Rohtak	1934	Mar.—May	121	Jan.—May 05	156	7
99	,,	1193	Mar.—June	30	April—June 05	54	9
93	,,	363	March	1	April 05	3	—
35	Sampla	2231	Mar.—May	175	Mar.—June 05	137	9
134	Jhaggar	296	June	1	Mar.—April 05	18	—
140	,,	231	May	2	Jan. 05	4	—
10	,,	637	Mar.—May	9	April—May 05	20	10
153	,,	572	Mar.—April	20	April—May 05	16	11
75	,,	645	May—June	23	April—May 05	31	9
171	,,	3896	Feb.—May	108	Mar.—May 05	101	9
135	,,	3602	Feb.—April	13	Nov. 04—Mar. 05	160	6
194	,,	2469	Jan.—Feb.	8	April—June 05	84	13
93	,,	956	March	1	April—June 05	36	—
155	,,	327	Mar.—May	23	April 05	53	10
81	Sampla	598	Feb.—May	61	Aug.—Sept. 04	12	2
113	Rohtak	609	Jan.—May	26	April—May 05	38	10
107	,,	4279	Feb.—June	204	April—May 05	19	9
108	,,	4076	Mar.—July	296	Jan.—May 05	21	5
96	,,	663	April	16	Mar.—May 05	60	10
15	,,	511	Feb.—April	62	April—May 05	50	11
65	,,	1285	Mar.—May	25	Mar.—April 05	39	9
32	,,	4191	Nov.—May	333	Feb.—May 05	315	8
33	,,	759	Feb.—May	24	April—June 05	18	10
31	,,	2463	Mar.—June	180	Mar.—June 05	162	8
53	,,	5024	April—May	15	Nov. 04—Mar. 05	98	5
28	,,	7824	Nov.—Dec.	6	Jan.—May 05	441	12
49	,,	4074	Jan.	1	Jan.—May 05	208	—
68	,,	1865	May	1	Mar.—May 05	139	—
31	Sampla	2164	April	1	Jan.—June 05	199	—
74	,,	5060	April—June	75	Sept. 04—June 05	334	2
12	,,	3765	March	1	Jan.—Mar. 05	224	—
95	,,	706	April—May	13	Feb.—April 05	29	8
68	,,	1887	April	3	Jan.—May 05	117	—
83	Gohana	4013	April	1	April—May 05	31	—
161	Jhaggar	1432	March	1	Mar.—May 05	90	—
60	,,	1031	April	3	Feb.—May 05	43	—
62	,,	12,227	Feb.—May	42	Sept.—Dec. 04	274	—
112	Rohtak	9723	Nov.—June	525	Oct. 04—May 05	208	3
85	,,	20,024	Feb.—May	12	Oct. 04—May 05	900	4
35	,,	7640	Mar.—June	262	Jan.—May 05	265	6
21	,,	3783	March	1	April—May 05	71	—
93	Sampla	5316	Mar.—June	100	Sept. 04—June 05	324	2
29	Gohana	1035	May—June	31	April—May 05	38	9
32	Jhaggar	1104	April—May	6	Oct. 04—June 05	59	4
128	,,	603	April	1	April 07	1	—
18	,,	1298	Nov.	1	Mar.—April 07	2	—
62	Sampla	1415	April	1	Feb.—May 07	91	—
72	,,	766	April—May	3	May 07	15	—
98	Rohtak	1494	June	1	April—June 07	32	—
63	Jhaggar	702	Feb.	1	April—June 07	26	—
114	Rohtak	519	Jan.—Mar.	47	April—May 07	5	35

TABLE XXVIII.

Shows the period during the epidemic of 1904—05 *in which* 31 *villages in Rohtak District infected the previous epidemic first reported plague deaths.*

	1904					1905				
	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May
Number of villages infected in 1903—04 in which deaths were returned in 1904—05.	1	3	3	2	0	4	2	5	11	0
Total number of villages which returned deaths each month.	2	6	9	17	23	63	101	156	232	208

TABLE XXIX.

Shows the interval for which 31 *villages in Rohtak District infected in the epidemics of* 1903—04 *and* 1904—05 *did not report plague deaths.*

	Number of months in which no plague deaths were returned												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Number of villages	0	3	2	2	2	2	1	3	7	5	2	1	1

TABLE XXX.

Data referring to villages in Rohtak District infected in epidemic of 1905—06.

			Details of infection of 1905—06		Details of infection of 1906—07		
No. of village	Tehsil	Population	Months during which deaths were returned	No. of deaths	Months during which deaths were returned	No. of deaths	No. of months free from deaths
2	Gohana	2269	April—May 06	110	May—June 07	64	10
12	,,	1205	Feb.—May 06	172	May—June 07	23	11
45	,,	1291	Feb.—April 06	53	March—May 07	117	10
79	,,	1720	March—April 06	17	April—June 07	116	11
83	Rohtak	4727	April—May 06	3	Jan.—May 07	266	—
16	Gohana	7509	April—June 06	195	Feb.—June 07	609	7
33	,,	1521	March—May 06	68	March—May 07	175	9
47	,,	2470	April—May 06	39	Nov. 06—May 07	177	5
3	Rohtak	2948	April—May 06	6	April—July 07	93	10
15	Gohana	4068	Feb.—May 06	258	March—June 07	265	9
3	,,	2443	Dec. 05—April 06	68	May—June 07	26	12
31	,,	4241	May—June 06	55	Sept. 06—July 07	897	2
49	,,	4568	Feb.—May 06	337	Feb.—June 07	258	8
39	Rohtak	3838	April 06	4	Feb.—May 07	135	—
39	Gohana	5657	April—June 06	32	Oct. 06—July 07	245	3
11	Rohtak	5126	April—May 06	13	Aug. 06—June 07	234	2
48	Gohana	4115	May 06	11	Feb.—May 07	274	8
80	,,	2245	May 06	8	March—April 07	174	9
13	,,	3185	Jan.—March 06	35	April—June 07	206	10
112	Rohtak	9723	Jan.—May 06	257	March—June 07	65	9
85	,,	20,024	April—May 06	3	Feb.—June 07	463	—
93	Sampla	5316	April 06	1	Feb.—June 07	416	—
35	Rohtak	7640	Feb.—May 06	9	March—June 07	49	10
29	Gohana	1035	Jan. 06	2	April—June 07	17	—
32	Jhaggar	1104	April 06	1	April—May 07	43	—
21	Rohtak	3783	Jan.—March 06	29	March—June 07	574	11
11	Jhaggar	645	April—May 06	5	Nil	—	—
94	Rohtak	1735	April—May 06	38	Nil	—	—
106	,,	1214	May 06	4	Nil	—	—
104	Sampla	266	Oct. 05—Feb. 06	82	Nil	—	—

TABLE XXXI.

Shows the period during the epidemic of 1906—07 *in which* 20 *villages in Rohtak District infected the previous epidemic first reported plague deaths.*

	1906					1907				
	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May
Number of villages infected in 1905—06 in which deaths were returned in 1906—07.	1	1	1	1	0	0	3	7	3	3
Total number of villages which returned deaths each month.	1	4	7	9	11	21	49	132	198	199

TABLE XXXII.

Shows the interval for which 20 *villages in Rohtak District infected in the epidemics of* 1905—06 *and* 1906—07 *did not report plague deaths.*

	Number of months in which no plague deaths were returned												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Number of villages	0	2	1	0	1	0	1	2	4	5	3	1	0

TABLE XXXIII.

Data referring to villages in Mozuffarnagar District infected in 1902—03 *epidemic as regards subsequent infection.*

Name	Population	Thana	Details of infection of 1902—03		Details of next infection		No. of months free
			Dates of first and last deaths	No. of deaths	Dates of first and last deaths	No. of deaths	
Chandan	97	Purkazi	30. 4. 03— 1. 5. 03	3	Nil	—	—
Yarpur	882	Thana-Bhavan	22. 3. 03—28. 3. 03	3	Nil	—	—
Sitheri	441	Budhana	5. 4. 03— 5. 4. 03	1	Nil	—	—
Mandkali	644	,,	4. 11. 02—28. 4. 03	59	3. 10. 04—14. 10. 03	10	17
Atawa	1445	,,	10. 2. 03—10. 2. 03	1	5. 11. 04—15. 11. 04	15	20
Barkali	561	,,	29. 11. 02—29. 11. 02	1	1. 5. 07— 7. 5. 07	15	53
Kherichangaim	779	Jansath	10. 5. 03—10. 5. 03	1	12. 5. 07—27. 5. 07	19	47
Tagain	681	Khatauli	14. 4. 03— 8. 5. 03	70	6. 2. 04—10. 2. 04	9	8
Chand-Samand	1163	,,	21. 4. 03—27. 4. 03	1	29. 3. 04— 7. 4. 04	27	10
Budma-Kalan	1742	Titavi	12. 3. 03—12. 3. 03	1	30. 2. 05—13. 5. 05	32	23
Basehra	4497	Purkazi	16. 4. 03— 8. 6. 03	69	12. 3. 05—31. 5. 05	172	20
Kadargarh	618	Thana-Bhavan	16. 9. 03—16. 9. 03	1	2. 1. 05—18. 3. 05	15	15
Majahadpur	1113	Khatauli	10. 4. 03— 2. 5. 03	16	14. 1. 05—15. 2. 05	57	19
Budhana	6664	Budhana	7. 3. 03— 7. 3. 03	1	21. 5. 05— 4. 6. 05	199	25
Bitanda	2663	,,	22. 11. 02—26. 4. 03	279	1. 9. 04— 3. 11. 04	30	16
Chandheri	694	,,	29. 11. 02—29. 11. 02	2	9. 5. 05—13. 5. 05	16	29
Kurthal	1823	,,	7. 4. 03—12. 5. 03	46	1. 10. 04—12. 10. 04	7	16
Baroda	2754	,,	4. 11. 02— 4. 11. 02	1	25. 4. 05—11. 6. 05	203	28
Khatauli	8695	Khatauli	13. 3. 03—10. 6. 03	11	23. 10. 03— 6. 4. 04	305	3
Ladpur	855	,,	12. 4. 03—12. 4. 03	1	8. 2. 04—29. 2. 04	37	9
Kalanda-Kalan	2140	,,	22. 4. 03—30. 5. 03	2	14. 11. 03—20. 11. 03	4	5
Meranpur	7209	Meranpur	26. 2. 03—26. 5. 03	150	31. 3. 04— 8. 6. 04	151	9
Kheri-Sarai	1443	,,	18. 4. 03—23. 5. 03	23	18. 4. 04—21. 4. 04	2	10
Amarpur	2294	Shahpur	10. 4. 03—29. 5. 03	21	11. 3. 04— 8. 5. 04	46	9
M. Nagar	23,444	M. Nagar	21. 2. 03—11. 6. 03	25	25. 10. 03—23. 4. 04	331	3

TABLE XXXIV.

Showing the interval during which 11 *villages in Mozuffarnagar District infected in* 1902—03 *and again in* 1903—04 *or subsequent epidemic did not report plague deaths.*

	Number of months during which no plague deaths were returned											
	1	2	3	4	5	6	7	8	9	10	11	12 & over
Number of villages	0	0	2	0	0	0	0	1	2	1	0	5

TABLE XXXV.

Showing the period during the epidemic of 1903—04 *in which* 6 *villages in Mozuffarnagar District infected the previous epidemic first reported plague deaths.*

	1903					1904				
	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May
Number of villages infected in 1902—03.	0	0	2	0	0	0	1	2	1	0
Total number of villages which returned deaths.	0	0	2	5	7	11	30	51	91	52

TABLE XXXVI.

Data referring to villages in Mozuffarnagar District infected in 1905—06 *as regards subsequent infection.*

			Details of infection in 1905—06		Details of next infection		
Name	Population	Thana	Dates of first and last deaths	No. of deaths	Dates of first and last deaths	No. of deaths	No. of months free
M. Nagar	23,444	M. Nagar	8. 3. 06—11. 6. 06	112	5. 11. 06—23. 5. 07	480	4
Maulaheri	915	,,	21. 3. 06—27. 4. 06	9	21. 3. 07—19. 4. 07	36	10
Chandpur	1109	,,	4. 4. 06—20. 5. 06	12	30. 11. 06— 3. 2. 07	41	5
Pinna	2853	Titawi	22. 4. 06—24. 4. 06	3	26. 3. 07—18. 6. 07	135	10
Saidpurah	308	,,	22. 4. 06—22. 4. 06	1	8. 2. 07— 6. 3. 07	26	9
Harsauli	3069	,,	21. 1. 06—23. 4. 06	208	25. 3. 07—23. 5. 07	348	10
Bodhena Kurd	1046	,,	16. 3. 06—13. 4. 06	32	8. 3. 07— 5. 4. 07	53	10
Nirmani	1315	,,	11. 2. 06—11. 2. 06	1	5. 4. 07—24. 5. 07	141	13
Ghesa Khera	571	Charthawal	4. 2. 06— 4. 2. 06	1	Nil	—	—
Kutesra	3565	,,	5. 11. 05—23. 1. 06	140	8. 3. 07— 5. 6. 07	273	13
Debchand	557	,,	3. 5. 06— 3. 5. 06	1	5. 5. 07—12. 5. 07	12	11
Kasauli	1323	,,	9. 4. 06—11. 5. 06	36	20. 9. 06—20. 9. 06	1	3
Jauli	2579	Bhopa	27. 3. 06— 5. 6. 06	53	10. 11. 06—21. 5. 07	103	4
Belra	1809	,,	22. 3. 06— 6. 6. 06	100	24. 1. 07—17. 5. 07	128	6
Teora	2699	,,	10. 6. 06—10. 6. 06	1	1. 12. 06— 1. 12. 06	1	5
Bhukareri	6316	,,	8. 2. 06—13. 5. 06	356	20. 2. 07—23. 5. 07	295	8
Sikri	3026	,,	6. 5. 06—23. 5. 06	24	7. 11. 06—24. 4. 07	47	5
Gadla	1767	,,	3. 2. 06— 9. 5. 06	105	4. 3. 07—15. 5. 07	117	9
Chaurawala	1543	,,	15. 5. 06—23. 5. 06	4	21. 10. 06— 5. 11. 06	16	4
Allahabas	282	,,	4. 3. 06— 4. 3. 06	1	Nil	—	—
Shamli	7478	Shamli	24. 12. 05—17. 5. 06	96	5. 2. 07— 7. 6. 07	239	8
Karmali	1078	,,	10. 12. 05— 5. 4. 06	59	17. 1. 07—28. 3. 07	86	8
Lank	3863	,,	10. 2. 06— 9. 5. 06	190	28. 2. 07— 3. 6. 07	206	8
Balra	2503	,,	10. 2. 06—28. 5. 06	180	3. 4. 07— 3. 5. 07	162	10
Bhaju	2563	,,	12. 4. 06—12. 4. 06	4	22. 3. 07— 3. 6. 07	218	10
Lilohan	1765	,,	17. 3. 06—17. 3. 06	1	Nil	—	—
Karandah	1471	,,	5. 4. 06— 5. 4. 06	1	27. 4. 06— 7. 6. 07	128	11
Chaunsa	1088	,,	22. 3. 06—22. 3. 06	4	30. 5. 07—30. 5. 07	1	13
Bauchra	738	,,	5. 5. 06—25. 5. 06	30	23. 5. 07— 5. 6. 07	38	11
Kheri	2212	,,	2. 4. 06— 2. 4. 06	5	15. 5. 07— 3. 6. 07	38	12
Bahri	2438	,,	14. 3. 06— 5. 6. 06	98	3. 3. 07— 2. 6. 07	224	8
Haranwara	1581	Thana-Bhavan	15. 3. 06—25. 4. 06	47	28. 3. 07—13. 5. 07	123	10
Mustgarh	359	,,	21. 1. 06—23. 1. 06	6	13. 4. 07— 6. 5. 07	50	14
Thanabhawan	8861	,,	24. 4. 06—12. 5. 06	13	13. 2. 07— 7. 6. 07	719	8
Bhatu	356	Jhinjhana	1. 4. 06—28. 4. 06	13	28. 5. 07— 3. 6. 07	12	12
Bela Mazra	771	Chausana	10. 11. 05—13. 12. 05	11	27. 3. 07— 8. 5. 07	134	14
Dathera	1245	,,	13. 12. 05—13. 12. 05	1	7. 4. 07— 8. 6. 07	32	15
Kairana	19,304	Kairana	19. 4. 06—25. 4. 06	2	14. 2. 07—13. 6. 07	2136	9
Gogwan	1158	,,	28. 4. 06—28. 5. 06	32	7. 5. 07—21. 6. 07	67	11

TABLE XXXVI (*continued*).

			Details of infection in 1905—06		Details of next infection		
Name	Population	Thana	Dates of first and last deaths	No. of deaths	Dates of first and last deaths	No. of deaths	No. of months free
Bharu	2694	Kairana	28. 5. 06—28. 5. 06	2	13. 4. 07— 3. 6. 07	189	10
Jansath	6507	Jansath	13. 8. 05—11. 6. 06	90	1. 12. 06— 8. 5. 07	268	5
Talra	1214	,,	19. 4. 06— 1. 5. 06	2	21. 12. 06— 3. 5. 07	169	6
Kawal	4268	,,	17. 3. 06—29. 5. 06	117	25. 1. 07— 5. 5. 07	295	7
Chitaura	1762	,,	24. 4. 06—15. 5. 06	23	23. 12. 06— 4. 6. 07	101	6
Kheri Ferozabad	983	,,	28. 3. 06—20. 5. 06	31	25. 4. 07— 8. 6. 07	145	10
Nagla Mubarik	538	,,	17. 4. 06—19. 5. 06	19	28. 4. 07— 3. 5. 07	21	10
Kamehra	1197	,,	3. 11. 05—15. 1. 06	53	14. 2. 07—13. 5. 07	171	12
Khilwara	416	,,	20. 3. 06— 5. 5. 06	41	28. 2. 07— 8. 3. 07	20	8
Bera Asa	1349	,,	14. 4. 06—23. 4. 06	2	23. 4. 07— 3. 6. 07	48	11
Baupara	1341	Khatauli	6. 4. 06—20. 4. 06	31	7. 4. 07— 3. 6. 07	166	11
Pur Bahani	4489	,,	2. 3. 06—16. 5. 06	128	3. 4. 07—30. 5. 07	247	10
Pura	805	,,	2. 2. 06—31. 5. 06	127	15. 1. 07— 7. 6. 07	80	7
Sandhera	1881	Meranpur	9. 4. 06— 6. 5. 06	59	2. 3. 07— 3. 4. 07	85	9
Tiraula	1286	,,	17. 5. 06—28. 5. 06	18	4. 3. 07— 7. 4. 07	58	9
Bhuma	1892	,,	17. 11. 05—17. 11. 05	1	27. 1. 07— 3. 6. 07	204	13
Mandwara	607	Budhana	4. 2. 06—14. 3. 06	54	1. 3. 07— 7. 5. 07	146	11
Warli	804	,,	23. 3. 06—21. 4. 06	27	28. 5. 07—28. 5. 07	2	12
Bahsana	201	,,	28. 3. 06—26. 4. 06	52	23. 4. 07— 3. 6. 07	24	11
Habibpur	825	,,	30. 3. 06— 8. 4. 06	24	22. 4. 07—12. 5. 07	47	11
Sarai	1148	,,	30. 3. 06—12. 5. 06	40	19. 4. 07— 3. 6. 07	49	10
Tanda Mazra	1086	,,	5. 4. 06—14. 5. 06	38	8. 4. 07— 3. 6. 07	48	10
Kandhla	11,563	Kandhla	14. 4. 06—25. 5. 06	52	20. 7. 06—14. 11. 06	9	1
Phagana	3236	,,	11. 5. 06—11. 5. 06	2	6. 6. 07— 6. 6. 07	2	12
Rampur Kheri	698	,,	13. 4. 06—13. 4. 06	6	Nil	—	—
Gujarpur	328	,,	21. 4. 06—21. 4. 06	3	Nil	—	—
Ailani	3796	,,	24. 4. 06—27. 4. 06	8	Nil	—	—
Khandrauli	2815	,,	11. 5. 06—11. 5. 06	4	15. 5. 07—17. 6. 07	36	11
Garhi Ram	1957	,,	19. 3. 06— 5. 4. 06	20	Nil	—	—
Loi	1905	,,	30. 10. 05—11. 12. 05	22	16. 3. 07— 7. 6. 07	114	14

TABLE XXXVII.

Showing the interval in months during which 53 *villages in Mozuffarnagar District infected in* 1905—06 *and again in* 1906—07 *did not report plague deaths.*

Number of months	0	1	2	3	4	5	6	7	8	9	10	11	12 & over
Number of villages	0	1	0	0	3	3	3	2	7	4	13	8	9

TABLE XXXVIII.

Showing the period during the epidemic of 1906—07 *in which* 53 *villages in Mozuffarnagar District infected in the previous epidemic first returned plague deaths.*

	1906						1907					
	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June
Number of villages infected in 1905—06.	1	0	0	1	4	3	5	7	13	12	6	1
Total number of villages which returned deaths.	1	1	1	7	16	35	77	159	288	438	427	169

TABLE XXXIX.

Data of future plague infection of villages in Rohtak District infected at the end of each epidemic.

No. of village	Tehsil	Population	No. of deaths during epidemic	Month when last death took place	Date when next infected	No. of months free from deaths
			A. End of epidemic of 1903—04.			
108	Rohtak	4076	296	July 04	Jan. 05	5
35	,,	7640	262	June 04	Jan. 05	6
31	,,	2463	180	,,	Mar. 05	8
99	,,	1193	30	,,	April 05	9
107	,,	4279	204	,,	April 05	9
112	,,	9723	519	,,	Oct. 04	3
29	Gohana	1035	31	,,	April 05	9
93	Sampla	5316	100	,,	Sept. 04	2
74	,,	5060	75	,,	Sept. 04	2
75	Jhaggar	645	23	,,	April 05	9
			B. End of epidemic of 1904—05.			
79	Rohtak	1686	16	June 05	Mar. 07	20
33	,,	759	18	,,	Mar. 07	20
31	,,	2463	162	,,	April 07	21
23	,,	413	15	,,	Nil	—
39	,,	3838	154	,,	April 06	9
3	,,	2948	83	,,	April 06	9
35	,,	200	20	,,	Nil	—

TABLE XXXIX (*continued*).

No. of village	Tehsil	Population	No. of deaths during epidemic	Month when last death took place	Date when next infected	No. of months free from deaths
			B. End of epidemic of 1904—05 (*continued*).			
30	Gohana	3327	231	,,	Mar. 07	20
82	,,	1928	112	,,	April 07	21
33	,,	1521	110	,,	Mar. 06	8
47	,,	2470	100	,,	April 06	9
21	,,	1296	76	,,	Feb. 07	19
15	,,	4068	20	,,	Feb. 06	7
3	,,	2443	23	,,	Jan. 06	6
31	,,	4241	31	,,	April 06	9
6	,,	3310	452	,,	Feb. 07	19
28	,,	330	28	,,	Feb. 07	19
74	,,	1923	15	,,	Mar. 07	20
78	,,	930	106	,,	June 07	23
66	,,	1467	28	,,	April 07	21
36	,,	2735	148	,,	Mar. 07	20
32	,,	1113	26	,,	April 07	21
11	,,	1191	78	,,	Nil	—
65	,,	1623	19	,,	April 07	21
67	,,	3133	187	,,	Mar. 07	20
64	,,	2034	211	,,	April 07	21
4	,,	2415	121	,,	April 07	21
48	,,	4115	332	,,	Mar. 06	8
13	Gohana	3185	61	June 05	Jan. 06	6
19	Sampla	1431	176	July	April 07	20
33	Jhaggar	4006	39	June	May 07	22
181	,,	974	72	,,	Nil	—
19	,,	927	2	,,	Nil	—
144	,,	1181	61	,,	Nil	—
32	,,	1104	47	,,	April 07	21
194	,,	2469	84	,,	Nil	—
93	,,	956	36	,,	Nil	—
			C. End of epidemic of 1905—06.			
83	Rohtak	4727	3	May	Jan. 07	7
35	,,	7640	9	,,	Mar. 07	9
94	,,	1735	38	,,	Nil	—
11	,,	5126	13	,,	Jan. 07	7
106	,,	1214	4	,,	Nil	—
3	,,	2948	6	,,	April 07	10
112	,,	9723	257	,,	Mar. 07	9
16	Gohana	7509	195	June	Feb. 07	7
2	,,	2269	110	,,	May 07	10
31	,,	4241	55	,,	Sept. 06	2
39	,,	5657	32	,,	Oct. 06	3
11	Jhaggar	645	5	May	Nil	—

TABLE XL.

Data referring to villages in Mozuffarnagar District infected at the end of each epidemic.

Name	Population	Thana	No. of deaths during epidemic	Month in which last death occurred	Date when next infected, month of first death	No. of months free
I. 1902—1903.						
Mozuffarnagar	23,444	M. Nagar	25	June 03	Oct. 03	3
Basera	4497	Purkazi	69	,,	Mar. 05	20
Khatauli	8695	Khatauli	4	,,	Oct. 03	3
Amarpur	2294	Shahpur	21	May 03	Mar. 04	9
Chandan	97	Purkazi	3	,,	Nil	—
Tigain	681	Khatauli	70	,,	Feb. 04	8
Majahadpur	1113	,,	16	,,	Jan. 05	19
Meranpur	7209	Meranpur	150	,,	Mar. 04	9
Kheri-Sarai	1443	,,	23	,,	April 04	10
Kailavda Kalan	2140	Khatauli	1	,,	Nov. 03	5
Karthal	1823	Budhana	46	,,	Oct. 04	17
II. 1903—1904.						
Tijalhera	2385	Purkazi	120	June 04	July 04	0
Gadla	1767	Bhopa	2	,,	Jan. 05	6
Belra	1809	,,	68	,,	July 04	0
Berahthra	1061	,,	25	,,	May 07	34
Tisa	3384	,,	29	,,	Sept. 04	2
Tandhera	1881	Meranpur	72	,,	Mar. 05	8
Sambalhera	2329	,,	93	,,	Nov. 04	4
Meranpur	7209	,,	151	,,	Aug. 04	1
Nayagind	1020	,,	2	,,	Mar. 07	32
Katka	830	Jansath	5	,,	Jan. 05	6
Kambera	1197	,,	40	,,	Nov. 05	16
Mubarik	538	,,	26	,,	April 06	21
Kawal	4268	,,	209	,,	Nov. 04	4
Pal	477	,,	4	,,	Feb. 07	31
Mahalki	1365	,,	40	,,	Mar. 05	8
Ahrora	484	,,	38	,,	Mar. 05	8
Gangdheri	1085	Khatauli	10	,,	Jan. 07	30
Kilaoda Kalan	2140	,,	2	,,	Dec. 04	5
Sudhan	510	,,	5	,,	Mar. 05	8
Lank	3863	Shamli	263	,,	Feb. 06	19
Balva	2503	,,	63	,,	Nov. 04	4
Purbalin	4489	Khatauli	67	,,	Dec. 04	5
III. 1904—1905.						
Rahkra	1425	Bhopa	34	June 05	Feb. 07	19
Badharwala	343	M. Nagar	1	,,	May 07	22
Medpur	221	,,	1	,,	Nil	—

TABLE XL (*continued*).

Name	Population	Thana	No. of deaths during epidemic	Month in which last death occurred	Date when next infected, month of first death	No. of months free
III. 1904—1905 (*continued*).						
Sherpur	1156	M. Nagar	2	June 05	Mar. 07	20
Nirana	680	,,	20	,,	Mar. 07	20
Jaranda	1744	,,	26	,,	April 07	21
Berahasa	1349	Jansath	3	,,	April 06	9
Sekheri	1207	,,	5	,,	Feb. 07	19
Mahmmadpur	600	Khatauli	5	,,	April 07	21
Satheri	1828	,,	33	,,	Feb. 07	19
Sohangni	1827	,,	100	,,	April 07	21
Karandah	1532	Titawi	32	,,	Nil	—
Jafarpur	756	Thanabhawan	26	,,	Feb. 07	19
Jalabad	6822	,,	319	,,	Jan. 07	18
Nogal	276	,,	7	,,	April 07	21
Chirdeka	509	,,	23	,,	April 07	21
Babri	2438	Shamli	276	,,	Mar. 06	8
Banat	3590	,,	256	,,	Mar. 07	20
Titaauli	1180	,,	156	,,	Mar. 07	20
Shamli	7478	,,	149	,,	Dec. 05	5
Kaserwa Khurd	914	,,	47	,,	May 07	22
Phagana	3236	Kandhla	30	,,	May 06	10
Gangani	6401	,,	157	,,	Mar. 07	20
Parasauli	2198	,,	114	,,	Nil	—
Kharar	3385	Budhana	143	,,	April 07	21
Bari	1199	,,	25	,,	Nil	—
Raipur	552	,,	54	,,	Nil	—
Jaula	4691	,,	163	,,	May 07	22
Budhana	6664	,,	199	,,	Jan. 07	18
Baranda	2754	,,	203	,,	April 07	21
Karthal	1823	,,	81	,,	April 07	21
Atawa	1445	,,	96	,,	Nil	—
Nagwa	1859	,,	114	,,	Nil	—
Kairana	19,304	Kairana	94	,,	April 06	9
Un	4502	Jhanjnana	272	,,	April 07	21
IV. 1905—1906.						
Belra	1809	Bhopa	100	June 06	Jan. 07	6
Jauli	2579	,,	53	,,	Nov. 06	4
Teora	2699	,,	1	,,	Dec. 06	5
Jansath	6507	Jansath	69	,,	Dec. 06	5
Mozuffarnagar	23,444	M. Nagar	112	,,	Nov. 06	4
Bahori	2323	Shamli	96	,,	Mar. 07	8

TABLE XLI.

Data referring to villages in the Amritsar District infected at the end of each epidemic.

No.	Tehsil	No. of deaths in epidemic	Month when last death occurred	Month when next death occurred	Months remained free
Epidemic of 1901—1902.					
57	Amritsar	42	July 02	April 03	8
143	,,	35	,,	Jan. 03	5
63	,,	2	,,	Nov. 03	14
241	,,	6	,,	May 03	9
113	,,	249	,,	Aug. 02	0
49	Tarn-Tarn	14	,,	Nov. 02	3
64	,,	70	,,	Feb. 03	6
170	Amritsar	130	June 02	Jan. 03	6
130	,,	12	,,	May 03	10
171	,,	41	,,	May 03	10
86	,,	25	,,	Jan. 03	6
196	,,	50	,,	Jan. 04	18
145	,,	21	,,	April 04	21
311	,,	140	,,	May 03	10
142	,,	31	,,	Jan. 07	54
77	,,	53	,,	Nov. 04	27
24	,,	3	,,	Feb. 04	19
97	Tarn-Tarn	27	,,	Mar. 03	8
213	,,	4	,,	April 03	9
34	,,	3	,,	April 04	21
46	,,	14	,,	Nov. 02	4
42	,,	2	,,	Nov. 02	4
19	,,	19	,,	April 04	21
67	,,	6	,,	Jan. 03	6
100	,,	7	,,	April 04	21
Epidemic of 1902—1903.					
189	Amritsar	20	July 03	Nil	—
213	,,	15	,,	April 04	8
210	,,	12	,,	April 04	8
219	,,	13	,,	June 04	10
164	,,	30	,,	Dec. 04	16
266	,,	40	,,	Jan. 05	17
334	,,	10	,,	Jan. 05	17
200	,,	20	,,	Feb. 05	18
253	,,	40	,,	Jan. 05	17
27	,,	40	,,	Feb. 04	6
137	,,	32	,,	Dec. 04	16
288	,,	26	,,	May 04	9
342	,,	25	,,	Feb. 05	18
113	,,	293	,,	Aug. 03	0

TABLE XLI (*continued*).

No.	Tehsil	No. of deaths in epidemic	Month when last death occurred	Month when next death occurred	Months remained free
		Epidemic of 1902—1903 (*continued*).			
323	Tarn-Tarn	27	July 03	Mar. 04	7
315	,,	185	,,	Mar. 05	19
233	,,	30	,,	Feb. 05	18
196	,,	195	,,	April 04	8
178	Ajnala	29	,,	Dec. 04	16
257	,,	50	,,	Dec. 04	16
160	,,	10	,,	Jan. 05	17
56	,,	25	,,	April 05	20
295	,,	25	,,	April 05	20
281	,,	100	,,	April 05	20
244	,,	3	,,	Nil	—
210	,,	70	,,	Oct. 04	14
276	,,	25	,,	May 07	45
161	,,	55	,,	May 04	9
131	,,	35	,,	Dec. 04	16
114	,,	8	,,	May 04	9
244–273	,,	90	,,	Mar. 04	7
267	,,	20	,,	Nil	—
		Epidemic of 1903—1904.			
232	Amritsar	111	July 04	Feb. 05	6
311	,,	326	,,	Dec. 04	4
5	,,	88	,,	Mar. 05	7
113	,,	1103	,,	Jan. 05	5
17	,,	233	,,	Jan. 05	5
112	Tarn-Tarn	135	,,	Feb. 05	6
42	,,	244	,,	April 05	8
143	,,	48	,,	Nil	—
240	,,	1	,,	Mar. 05	7
196	,,	85	,,	Mar. 05	7
270	Ajnala	138	,,	May 05	9
100	,,	62	,,	May 07	33
63	,,	16	,,	Mar. 07	31
91	,,	29	,,	April 07	32
		Epidemic of 1904—1905.			
287	Amritsar	134	July 05	April 06	8
113	,,	1073	,,	Aug. 05	0
17	,,	86	,,	April 06	8
5	Tarn-Tarn	101	,,	Mar. 06	7
142	,,	117	,,	April 07	20
267	,,	88	,,	June 06	10
300	,,	107	,,	April 07	20
241	,,	24	,,	Mar. 07	19

TABLE XLI (*continued*).

No.	Tehsil	No. of deaths in epidemic	Month when last death occurred	Month when next death occurred	Months remained free
		Epidemic of 1904—1905 (*continued*).			
243	Tarn-Tarn	18	July 05	Mar. 07	19
264	,,	256	,,	May 06	9
321	,,	140	,,	Nil	—
286	,,	95	,,	Feb. 07	18
66	,,	214	,,	Jan. 06	5
59	,,	9	,,	Nil	—
281	,,	77	,,	Jan. 07	17
23	,,	26	,,	Nil	—
150	,,	175	,,	April 07	20
240	,,	144	,,	Nil	—
319	,,	47	,,	May 06	9
64	,,	165	,,	May 06	9
254	,,	159	,,	April 07	20
268	,,	86	,,	June 06	10
311	,,	278	,,	April 06	8
76	,,	5	,,	April 06	8
179	,,	213	,,	Mar. 07	19
326	,,	35	,,	Feb. 07	18
26	Ajnala	213	,,	Mar. 07	19
269	,,	54	,,	April 07	20
295	,,	58	,,	Feb. 07	18
81	,,	90	,,	April 07	20
303	,,	52	,,	April 07	20
267	,,	130	,,	Feb. 07	18
206	,,	16	,,	Nil	—
262	,,	87	,,	April 07	20
226	,,	12	,,	April 07	20
309	,,	86	,,	April 07	20
161	,,	70	,,	Mar. 07	19
251	,,	86	,,	May 07	21
235	,,	11	,,	Nil	—
		Epidemic of 1905—1906.			
279	Amritsar	25	July 06	Dec. 06	4
160	,,	1	,,	Feb. 07	6
232	,,	70	,,	Feb. 07	6
262	,,	113	,,	Nil	—
311	,,	202	,,	Mar. 07	7
296	,,	9	,,	Nil	—
5	,,	39	,,	Jan. 07	5
113	,,	1863	,,	Aug. 06	0
17	,,	30	,,	Jan. 07	5
225	,,	113	,,	April 07	8
197	Ajnala	11	,,	Jan. 07	5

TABLE XLII.

Showing the interval of freedom from plague deaths enjoyed by villages in Rohtak District which were still infected at the end of the different epidemics.

	Number of months during which no plague deaths were returned												
	0	1	2	3	4	5	6	7	8	9	10	11	12 & over
1903—04.													
Number of villages	0	0	2	1	0	1	1	0	1	4	—	—	—
1904—05.													
Number of villages	0	0	0	0	0	0	2	1	2	4	0	0	20
1905—06.													
Number of villages	0	0	1	1	0	0	0	3	0	2	2	—	—

TABLE XLIII.

Showing the interval of freedom from plague deaths enjoyed by villages in the Mozuffarnagar District which were still infected at the end of the different epidemics.

	Number of months during which no plague deaths were returned												
	0	1	2	3	4	5	6	7	8	9	10	11	12 & over
1902—03.													
Number of villages	0	0	0	2	0	1	0	0	1	2	1	0	3
1903—04.													
Number of villages	2	1	1	0	3	1	2	0	4	0	0	0	8
1904—05.													
Number of villages	0	0	0	0	0	1	0	0	1	2	1	0	23
1905—06.													
Number of villages	0	0	0	0	2	2	1	0	1	0	0	0	0

TABLE XLIV.

Showing the interval of freedom from plague deaths enjoyed by villages in the Amritsar District which were still infected at the end of the different epidemics.

	Number of months during which no plague deaths were returned												
	0	1	2	3	4	5	6	7	8	9	10	11	12 & over
1901—02.													
Number of villages	1	0	0	1	2	1	4	0	2	2	3	0	9
1902—03.													
Number of villages	1	0	0	0	0	0	1	2	3	3	1	0	18
1903—04.													
Number of villages	0	0	0	0	1	2	2	3	1	1	0	0	3
1904—05.													
Number of villages	1	0	0	0	0	1	0	1	4	3	2	0	21
1905—06.													
Number of villages	1	0	0	0	1	3	2	1	1	—	—	—	—

TABLE XLV.

Showing the total number of villages in Rohtak District and the number of villages infected in each epidemic.

Total No. of villages	Number of villages infected			
	1st epidemic 1903—04	2nd epidemic 1904—05	3rd epidemic 1905—06	4th epidemic 1906—07
499	57	285	30	249

TABLE XLVI.

Showing the actual number of villages in Rohtak District which were infected in no, one, two, three and four epidemics.

Number of villages infected in				
No epidemic	One epidemic	Two epidemics	Three epidemics	Four epidemics
145	138	172	37	7

TABLE XLVII.

Showing the calculated probable number of villages in Rohtak District which would have been infected in no, one, two, three and four epidemics if all villages were equally liable to infection in all four epidemics.

Number of villages infected in				
No epidemic	One epidemic	Two epidemics	Three epidemics	Four epidemics
89	225	159	24	1

TABLE XLVIII.

Showing the total number of villages in Mozuffarnagar District and the number of villages infected in each epidemic.

Total No. of villages	Number of villages infected in				
	1st epidemic	2nd epidemic	3rd epidemic	4th epidemic	5th epidemic
973	25	130	313	69	579

TABLE XLIX.

Showing the actual number of villages in Mozuffarnagar District which were infected in no, one, two, three, four and five epidemics.

Number of villages infected in					
No epidemic	One epidemic	Two epidemics	Three epidemics	Four epidemics	Five epidemics
334	301	226	86	25	1

TABLE L.

Showing the calculated probable number of villages in Mozuffarnagar District which would have been infected in no, one, two, three, four and five epidemics if all villages were equally liable to infection in all five epidemics.

Number of villages infected in					
No epidemic	One epidemic	Two epidemics	Three epidemics	Four epidemics	Five epidemics
202	457	261	49	3	0·05

TABLE LI.

Showing the total number of villages in Amritsar District and the number of villages infected in each epidemic.

Total No. of villages	Number of villages infected in					
	1st epidemic	2nd epidemic	3rd epidemic	4th epidemic	5th epidemic	6th epidemic
1062	62	506	445	669	276	604

TABLE LII.

Showing the actual number of villages in Amritsar District which were infected in no, one, two, three, four, five and six epidemics.

Number of villages infected in						
No epidemic	One epidemic	Two epidemics	Three epidemics	Four epidemics	Five epidemics	Six epidemics
155	183	211	230	169	93	21

TABLE LIII.

Showing the calculated probable number of villages in Amritsar District which would have been infected in no, one, two, three, four, five and six epidemics if all villages were equally liable to infection in all six epidemics.

Number of villages infected in						
No epidemic	One epidemic	Two epidemics	Three epidemics	Four epidemics	Five epidemics	Six epidemics
48·5	195·8	329·1	295·1	148·8	40	4·5

TABLE LIV.

Showing the number of villages in Rohtak District infected each epidemic along with the total and average population.

Epidemic of	No. of villages infected	Total population of villages infected	Average population of villages infected
1903—1904	57	149,524	2623
1904—1905	285	493,145	1730
1905—1906	30	117,631	3921
1906—1907	249	471,670	1894

TABLE LV.

Showing the number of villages in Rohtak District with the total and average population infected in no, one, two, three and four epidemics.

	No. of villages	Total population of villages	Average population of villages
Never infected	145	63,051	435
Infected in one epidemic	138	109,491	793
Infected in two epidemics .	172	270,986	1575
Infected in three epidemics	37	128,669	3478
Infected in four epidemics	7	48,625	6946
Total	499	620,822	1244

TABLE LVI.

Showing the number of villages in Mozuffarnagar District infected each epidemic, with their mean population.

No. and year of epidemic		No. of villages infected	Total population of villages infected	Mean population of villages infected
1st	1902—03	25	75,341	3014
2nd	1903—04	130	257,091	1978
3rd	1904—05	313	532,941	1703
4th	1905—06	69	183,270	2656
5th	1906—07	579	744,948	1286

TABLE LVII.

Showing the number of villages in Mozuffarnagar District with their mean population infected in no epidemic, in only one epidemic, in any two, in any three, in any four and in all five epidemics.

	No. of villages	Total population of villages	Mean population of villages
No epidemic	334	127,217	381
One epidemic	301	211,861	704
Two epidemics	226	284,843	1260
Three epidemics	86	187,300	2178
Four epidemics	25	83,231	3329
Five epidemics	1	23,444	23,444
Total	973	917,896	942

TABLE LVIII.

Showing the number of villages in Amritsar District infected each epidemic, with their mean population.

No. and year of epidemic		No. of villages infected	Total population of villages infected	Mean population of villages infected
1st	1901—02	63	269,467	4277
2nd	1902—03	506	734,753	1452
3rd	1903—04	445	682,138	1533
4th	1904—05	669	869,853	1300
5th	1905—06	276	510,464	1849
6th	1906—07	604	813,116	1346

TABLE LIX.

Showing the number of villages in Amritsar District with their mean population, infected in no epidemic, in only one, in any two, three, four, five, and in all six epidemics.

Infected in	No. of villages	Total population of villages	Mean population of villages
No epidemic	155	39,225	253
One epidemic	183	83,779	458
Two epidemics	211	125,899	597
Three epidemics	230	210,281	914
Four epidemics	169	206,547	1222
Five epidemics	93	155,971	1677
Six epidemics	21	217,888	10,376
Total	1062	1,039,590	978

TABLE LX.

Showing in Rohtak District for each Tehsil the number of villages and their percentage on the total villages infected in no epidemic, and in one, two, three and four epidemics.

	Rohtak Tehsil	Gohana Tehsil	Sampla Tehsil	Jhaggar Tehsil
Total no. of villages	109	79	124	187
Total population	195,423	140,682	160,262	124,455
Average population per village	1793	1781	1292	666
No. of villages never infected	21	6	21	97
Percent. of villages never infected	19·3	7·6	16·9	51·9
No. of villages inf. 1 epidemic	28	13	36	61
Percent. of villages inf. 1 epidemic	25·7	16·5	29	32·6
No. of villages inf. 2 epidemics	39	47	61	25
Percent. of villages inf. 2 epidemics	35·8	59·5	49·2	13·4
No. of villages inf. 3 epidemics	17	12	5	3
Percent. of villages inf. 3 epidemics	15·6	15·1	4	1·6
No. of villages inf. 4 epidemics	4	1	1	1
Percent. of villages inf. 4 epidemics	3·7	1·3	0·9	0·5

Part II. STATISTICAL ANALYSES OF DATA RESPECTING EPIDEMICS OF PLAGUE IN THREE DISTRICTS OF THE PUNJAB.

By M. GREENWOOD, Jr.

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First Report—*On the factors which influence the frequency of infection.*

It was decided to proceed with the analysis of the data collected by Major Lamb in the following stages:—

1. To determine whether the numbers of villages attacked in any one, two or more epidemics were in agreement with the numbers one would expect to find supposing the matter were merely one of chance.
2. In the event of such distributions not proving to be random, to attempt to account for the non-random character, paying special attention to the supposed importance of size of village in this connection.
3. In the event of the distribution being non-random, and its character not being explained by variations in the populations, to investigate the matter further.
4. To consider and report upon the variations in percentages of deaths which were manifest even in villages of approximately the same size.

Since many of the points referred to are of considerable importance and their statistical treatment will be a somewhat lengthy process, it will make for clearness and avoid unnecessary delay if I embody my results in a series of separate reports. In the present communication, I shall deal with factors which influence the recurrence of infections, in so far as I have been able to reach statistical results. I desire also to remark that I have attempted on this occasion, and shall endeavour in any subsequent communications, to explain intelligibly the analytical methods I employ. I do not mean by this that I propose to discuss the somewhat difficult mathematical considerations from which the methods in question were, as a matter of history, derived; but merely that I hope to furnish sufficient explanation to place the reader in a position to judge for himself whether the processes employed and the conclusions stated are, or are not, justifiable.

The first point to be observed is that one must adopt some standard as to what constitutes an infected village. When, for instance, only a few cases of plague have been found in a large village, it may be that these have been imported and are no true indications of local morbidity. To decide this point is not the duty of a statistician, since it involves special knowledge only possessed by those familiar with local conditions. I have, for the purposes of the present inquiry, been instructed by those who have this knowledge to regard as an infected village any village from which a plague death has been reported; the effect of modifying this criterion may be matter for subsequent inquiry.

At the outset, it is apparent that the size of the village is an important factor in the likelihood of its being infected. This is almost axiomatic on any theory of aetiology and is abundantly demonstrated by the statistics of which Table I give merely a selection.

The next point is to consider whether this factor is alone responsible for the selective grouping of villages affected in various combinations of epidemics, but before examining this we must consider the analytical process to be used.

The problem is of the following nature—There are a villages in a district and plague infections have been reported in n different epidemics, c_1 in the first, c_2 in the second, and so on, up to c_n. We also know that a_n villages have been infected in all epidemics, a_{n-1} in $n-1$ of the epidemics, and so on. Supposing that the fact of being infected in any one year is quite independent of the fact of being affected in any other year, is the number of actual recurrences in agreement with what we should expect?

This problem may be approached in the following way. The chance of being affected in the first epidemic is $\frac{c_1}{a}$, of being affected in the second epidemic $\frac{c_2}{a}$, and so on. Therefore the chance of being affected in all epidemics is $\frac{c_1 c_2 c_3 \ldots c_n}{a^n}$ and the total number which should be so infected is $a \times \frac{c_1 c_2 c_3 \ldots c_n}{a^n}$. Thus the number which would be infected in the first three epidemics is $a \times \frac{c_1 c_2 c_3 d_4 d_5 \ldots d_n}{a^n}$ where

$$d_4 = a - c_4 \; d_5 = a - c_5 \text{ etc.}$$

And we shall find the number infected in any three epidemics by taking a times the sum of all such expressions as

$$\frac{c_1 c_2 c_3 d_4 d_5 d_6 \dots d_n}{a_n}.$$

In this way one obtains the most probable number of infections for each possible combination of epidemics and we next require some method of testing the agreement between the observed numbers and the calculated ones. Of the various tests which have been proposed the most satisfactory is that devised by Karl Pearson[1] which can, for our purposes, be looked at in the following simple way.

Supposing the observed value in any case is x and the value expected, on any hypothesis which we desire to test, y. Then $\frac{(x-y)^2}{y}$ will be a measure of agreement independent of sign and its summed value $S\frac{(x-y)^2}{y}$, for all the classes we have formed will be a measure of the total agreement. The problem now reduces itself to the following—How many times, on the average, will the observed values in a series of events which are known to follow a given law of distribution differ from the expected values owing to random sampling so as to produce a specified arithmetical value of $S\frac{(x-y)^2}{y}$?

This has been answered by Pearson in the memoir referred to and a table constructed by W. Palin Elderton[2].

For instance, suppose that in a certain case $S\frac{(x-y)^2}{y}$, or as it is usually termed χ^2, is equal to 10 and we find against this value for material grouped in the number of subdivisions used for our material, the fraction ·8. This means that if the law be true we should get, on repeating our observations, as bad an agreement as or a worse agreement than that given by our material, eight times out of ten. In other words it is most likely that the theoretical law assumed really describes the observations.

What value of P (the ·8 of my illustration is called P) should be taken as good evidence for the truth of one's hypothesis, is partly a matter of individual judgment and partly depends on the nature of the material examined. I should be disposed to regard any value of P greater than ·45 as good and greater than ·25 as fair evidence in such problems as those we have to consider now. I do not propose to examine the mathematical justification of the method I have outlined; reference can be made by those readers who are interested in the subject to the memoir of Pearson which I have cited. It is, I hope, sufficiently clear that the process lends itself to our particular investigation. Some practical difficulties now deserve notice.

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[1] "On the Criterion that a given System of Deviations from the Probable in the Case of a Correlated System of Variables is such that it can be reasonably supposed to have arisen from Random Sampling." K. Pearson, *Phil. Mag.*, 1900, Vol. L., p. 157.

[2] "Tables for Testing the Goodness of Fit of Theory to Observation." W. P. Elderton, *Biom.*, 1902, p. 155.

Although the calculation of the probable number of villages to be infected in any combination of epidemics is, as I have shown, merely a matter of simple arithmetic, yet the arithmetic becomes extremely laborious when the number of epidemics under review is even moderately large. Thus in Amritsar district there are six epidemics and the complete evaluation of the various combinations of villages affected never, once, twice etc., requires the determination of 64 distinct products each composed of six terms. Without a mechanical calculator this is an impracticable task and even with such help it is very tedious. It may be asked whether an approximation to the value cannot be obtained by some easier method and a way out of the difficulty might seem to be afforded by using a mean value. Thus if c_1, c_2, c_3 etc. were infected in each year we might determine the mean probability of infection

$$\frac{c_1 + c_2 + c_3 + \dots c_n}{na} = \text{say } \bar{p},$$

and, similarly, the mean probability of not being infected = say $\bar{q}$. Then the number which should be infected in any two years *e.g.* would be $a\ (\bar{p})^2 (\bar{q})^{n-2}$. The objections to this simplification are, I think, insuperable. The total number of epidemics under observation is absolutely small and the proportion of villages attacked from epidemic to epidemic, varies enormously. For instance, in Amritsar, out of 1062 villages—

62	were	attacked in	1901—2
506	„	„	1902—3
445	„	„	1903—4
669	„	„	1904—5
276	„	„	1905—6
604	„	„	1906—7.

The probable error of a mean determined from so short and variable a series is large (for *normally distributed variables,* the probable error of the mean is $\cdot 67449 \times \frac{\text{Standard Deviation}}{\sqrt{\text{Number of Observations}}}$).

The difference between the results obtained by calculating the probable numbers in both ways is appreciable as will be seen in Table II. I have therefore made all the calculations by the direct summation method already described. Although the process was very long it was rendered possible by the use of a large Brunsviga Calculator and all additions were checked with a Burroughs Adding Machine.

I shall give the complete working of one table, that for the whole Amritsar district. From the figures given above, we see that the chances of being infected in each year are:

$$p_1 = \cdot 0583804, \quad p_2 = \cdot 4764595, \quad p_3 = \cdot 4190207,$$
$$p_4 = \cdot 6299435, \quad p_5 = \cdot 2598870, \quad p_6 = \cdot 5687382,$$

and the corresponding chances of not being infected are:

$$q_1 = \cdot 9416196, \quad q_2 = \cdot 5235405, \quad q_3 = \cdot 5809793,$$
$$q_4 = \cdot 3700565, \quad q_5 = \cdot 7401130, \quad q_6 = \cdot 4312618.$$

With these values for the independent probabilities, we determine the expected number in each category. Thus the probable number affected five times is

$$1062\,(p_1p_2p_3p_4p_5q_6 + p_1p_2p_3p_4p_6q_5 + p_1p_2p_3p_5p_6q_4 + p_1p_2p_4p_5p_6q_3 + p_1p_3p_4p_5p_6q_2 + p_2p_3p_4p_5p_6q_1).$$

Finally we reach Table III the headings of which are explanatory. The value of P is so small that we can evidently not regard the distribution as a random one. Tables IV and V show similar results for the other two districts. Table III A gives the details for Amritsar.

We now come to the question whether the non-random character of these distributions is due to mixing together villages of very different sizes. I approached the problem in the following way. A series of groups was chosen, each containing villages falling within assigned limits of size. Each group was then treated in the manner just explained, the probability of infection in each epidemic being of course determined separately for each group. The results appear in Tables VI to XXV, the actual working details being omitted to save space. These tables merit somewhat close attention. In the first place it is obvious that the majority exhibit a much closer agreement between theory and observation than could be discerned in the general Tables III to V, but an objection will immediately occur to the reader. Tables VI to XXV are sub-samples and consequently include far fewer observations than figure in the Tables III to V, is this the cause of the better agreement?

It is clear that the test of agreement which I have employed gives an answer which in some measure depends on the absolute size of the experience. Thus our criterion is the value of

$$S\frac{(x-y)^2}{y}=\chi^2.$$

If we multiply x and y by a constant k this becomes:

$$S\frac{k^2(x-y)^2}{ky},$$

or

$$S\frac{k(x-y)^2}{y}.$$

Hence if k is a proper fraction χ^2 is diminished and the apparent goodness of fit increased. This is, of course, in accordance with the demands of common sense. A deviation of, say, ten per cent. from the theoretical value when we examine, say, a sample of 100 would not be regarded as making so much against the theory as a ten per cent. difference when the sample was one of 1000.

Now it seemed to me that one could form a reasonable opinion as to whether the higher values of P in some of the groups are merely due to reduction in absolute size and not to reduction in the relative discrepancy between theory and practice, quite simply. I have taken each table of totals (III—V) and determined the value which P would take if the relations between observed and calculated values were not disturbed but the value of χ^2 reduced to what would be given by smaller total numbers of observations.

In the case of Amritsar, the values of χ^2 reduced to 153, 129, 390 observations are still so large as to give values of P less than one in a billion; even with a total of 45 (the smallest Amritsar Group) and using five classes instead of six, P only rises to ·0001. The Mozuffarnagar total reduced to 72, 82, 141 and 161 observations also gives P less than one in a million. Rohtak gives $P=·04$ when the total is reduced to 44 and $P=·01$ for 60 observations. While I admit the test to be a rough one, I can see no valid objection to it. I hold it to be certainly true that the improved agreement between theory and practice shown in many of the Tables VI—XXV is not to any serious extent dependent on the reduction in numbers, as compared with Tables III—V alone. We are now in a position to assert that size of population is an important factor in determining whether a village is likely to be attacked by plague, and that elimination of this factor tends to bring the number of recurrent infections into agreement with a chance distribution, but a variety of collateral questions are raised by the analytical results. To begin with, there is not only a great difference in the results from the several districts, but between the returns for groups of the same district. Why is this? Taking the second point first, the most obvious explanation would seem to be that the groups are arbitrary and that the variations in size within each are not the same. There is no real reason to think that absolute size within the group has anything to do with the matter, because the *a priori* chance of being infected has been calculated separately for each group; it could only be a question of relative variation. I determined the mean population of each group, the mean square deviation from the mean, its square root (the Standard Deviation) and the percentage that the standard deviation was of the mean (the Coefficient of Variation). These constants are shown in Tables VI to XXV and are collected together in Table XXVI. Taken as a whole, there appears to be a *slight* tendency for the higher values of P to be associated with smaller coefficients of variation and this was to some extent confirmed by determining the correlation between the value of P and the size of the coefficients of variation. I cannot, however, attribute any real importance to these findings. The figures marked with an asterisk are hardly comparable with the others since the variation depends mainly on the inclusion of a few very large or very small villages or towns. But if we exclude these, the variation is rarely in excess of 10. Now the census from which the populations were obtained was taken in 1901 and I understand that a variety of local circumstances prevent the census returns being very accurate (as a basis for estimating the present population). I was advised that one could not safely regard the error as being much less than ten per cent. In other words the variation within the groups is of the same order as the error in the estimates of population and cannot serve as a basis for valid statistical deductions. I am, therefore, of opinion that we cannot attribute the differences in the values of P to this factor. I must not of course be understood to mean that differences in size-variation have not played a part, but merely as stating that we have no reliable evidence that such is the case.

A contributory influence of appreciably greater weight is the difference in number of villages within each group, especially as in some of the smaller groups fewer subdivisions were made owing to the calculated numbers of infections in five and six epidemics being very small. I have tested this in the same way as in comparing totals with groups (vid. supr.). Table XI gives $P = \cdot 07$ for 129 villages and this rises to ·57 for 44 observations. Table X gives $P = \cdot 148$ when we reduce the number of categories from 6 to 5 and the total to 44. Similarly, Table IX reduces to $P = \cdot 24$ and Table VIII to $P = \cdot 275$. Using the Mozuffarnagar results we find, reducing to a total of 44 and five categories (which enables us to compare with the Rohtak figures), Table XVI gives $P = \cdot 44$, Table XIV, $P = \cdot 08$, Table XV, $P = \cdot 21$.

These results show quite clearly that the size of the group and the number of sub-classes, whether 7, 6, or 5, employed, have had a considerable share in causing diversity within each district. They also show that the greatly improved fit among the Rohtak Groups as compared with Mozuffarnagar and especially Amritsar, is *partly*, but only partly accounted for, by the differences mentioned. We have seen that in one group only out of the Amritsar set have we been able to bring up the goodness of fit to the general level of the Rohtak returns. The same remark applies but with appreciably less force to a comparison between Mozuffarnagar and Rohtak. Table XXVIII contains all the Rohtak groups which were calculated in five classes reduced to a total of 44 and all the returns from the other districts reduced to the same dimensions. It will be found to support the preceding observations to the effect that Amritsar shows definitely less good agreement with expectation than do the other districts. The averages for each district are simple means formed without weighting with the numbers which actually appeared in each group; it is hardly possible to give truly comparable means because of the difficulty of assigning proper weights.

It therefore results, I think, that while careful allowance is to be made for the various sources of fallacy which are involved in attempting such comparisons as these, possibilities of error which I have not—consciously at least—minimised, there is an appreciable difference between the returns for any one district within that district and a still more appreciable difference between the returns for Amritsar on the one side and Rohtak on the other.

In the case of Rohtak, the general run of values for P is so good that we seem entitled to conclude that size of village has been by far the most important factor in producing the want of agreement which is seen in the general Table. Other factors may have contributed to the results but their importance would seem to have been relatively small.

In Mozuffarnagar more hesitation is appropriate, but the fit in some important cases is so good that we are still justified in assigning the pre-eminence to the size factor[1]. In Amritsar, although certainly one and possibly two examples prove that size has been of considerable importance, yet the general trend of the tables seem to force one to the conclusion that something else has had a special influence not to be discerned in the other districts. What this something else is, I do not see my way to deciding on the strength of the information before me. A study of the maps and of the extremely interesting written descriptions with which I was furnished appear to show certain differences. Rohtak appears to be less thickly peopled than Amritsar[2]; Amritsar is somewhat better served by means of communication and possesses much larger and more important towns. Nothing which I have been able to find in the report of the Commissioners or in such descriptions of plague epidemiology as I have consulted, has suggested anything capable of statistical analysis with respect to these points.

The following ideas have occurred to me. Amritsar is much the largest city in the three districts and its intercourse with other towns and villages in the district must be great even out of proportion to its size, since it is the centre of important administrative business and is of religious interest to the Sikhs. The main lines of migration to and from the city are presumably not random, just as we find in England that of two apparently equally convenient highways between large towns one is much the more often traversed. Villages lying along the habitual lines of travel might be specially prone to importation of sources of infection irrespective of their size or other local peculiarities. The difficulty of testing this statistically lies in the fact that even in Amritsar size plays a part in the recurrence or non-recurrence of infections, but still the matter might be tested and I will do so[3], unless those whose practical knowledge of the aetiological problem is far greater than mine, decide that the idea is baseless.

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[1] It may, perhaps, be asked why, in view of Table XXVIII, I class Mozuffarnagar rather with Rohtak than with Amritsar. The answer is that, apart from the rough nature of the test which Table XXVIII exemplifies, certain of the groups in Mozuffarnagar, notably Table XII with a relatively large number of included villages, exhibit a fairly close agreement between expectation and observation. In all the circumstances, I think this fact should have weight. The reader will of course draw his own conclusions.

[2] Rohtak ·3 per acre, Amritsar 1 per acre. I have no record of the area of Mozuffarnagar District.

[3] A preliminary analysis shows that the Amritsar villages within two miles of the line of railway are not a random sample of the total in respect to plague recurrence.

Another point which might be worth consideration is the possibility that the greater percentage of Sikh inhabitants, who are, I understand, more numerous in the Amritsar district than elsewhere, is of some significance.

One other conclusion seems to be supported by the statistical evidence. In investigating the arithmetical cause of the poor agreement between expectation and observation in Amritsar district, I was struck by the fact that the main discrepancy was often due to the calculated number of never-infected villages falling short of the observed number. To see whether this were really so, I constructed Table XXVII. It will be seen that in the majority of cases, notably in Amritsar, the observed excess in the first group has been largely responsible for the poor fit. It might be hastily assumed that this is due to taking an arbitrary standard of plague infection, viz. the occurrence of a single death. This is not necessarily or even probably the case. The number of such villages is not very large and were it large the *a priori* probability of non-infection would be greatly increased by including the villages with one or two deaths in the never-infected class. This would disturb the balance altogether and might conceivably make the agreement even worse. This is a point I can test, if the Committee so desire; as the case stands, I am disposed to conclude that certain villages, for reasons which do not appear in the mere statistics, are peculiarly difficult to infect.

Since the question as to whether a village infected in one year is, *ipso facto*, more likely to become infected in the next following year has a special bearing on the problem of importation as contrasted with recrudescence, I have investigated the point separately in the following way.

Six groups of Amritsar villages were chosen, viz.

(1) Villages of populations 1200—1400.
(2) „ „ 1000—1200.
(3) „ „ 800—1000.
(4) „ „ 500—700.
(5) „ „ 400—500.

The numbers infected in the years 1904, 1905, 1906, 1907, were ascertained and then the numbers expected to be attacked in any pair of consecutive years were calculated on the assumption of equal incidence within the groups.

The actual numbers were then compared with the calculated figures for each group and each pair of years. The goodness of agreement was then measured by the method used in other sections of this report. The results are collected in Table XXIX.

These results merit careful attention. It will be noticed that for the years 1904—5 and 1905—6, the agreement as measured by the value of P is extremely close, very fair for 1903—4, and moderate or poor (in view of the total number of observations) for 1906—7. The agreement obtained when the totals are used and grouped in years may be considered moderate.

On looking at the details, we notice that the agreement is, in general, worse in the groups within which the relative variations in size are largest. Thus the groups of big villages agree quite well in all cases but one and the poor agreement in the table for 1906—7 is mainly due to the group 500—700, while the fit for 1903—4 is appreciably diminished by the same group. It must, however, be carefully observed that this particular group is the largest and should have the most weight assigned to it. It must also be noticed that the actual numbers are, in the majority of cases, larger than the calculated ones and that where the agreement is best the likelihood of failure is least.

Let us next consider what sort of results we should expect to get, if we were to adopt an hypothesis as to epidemic origins.

If we hold that a plague outbreak depends on the recrudescence or bursting into activity of infective agencies left over from the last epidemic, then the following deductions are, in my opinion, inevitable. Only villages which have been attacked previously, in some shape or form, can *ex hypothesi* be attacked again. All those which have been so evidently infected as to report at least one death will be eligible for reinfection. In addition there will be some villages so slightly affected or placed under such unfavourable circumstances for the obtaining of exact information that they do not figure in our returns but which are eligible for attack in the following year. We should not, therefore, be surprised to find that *a very small proportion* of the villages which showed cases in any one year had not been *ostensibly* affected in the previous year. Unless, however, we assume that in two consecutive outbreaks the first is always much milder than the second, an assumption without evidence in its favour, the magnitude of this error cannot be sufficient to vitiate the general statement that the brunt of the second outbreak must be borne by villages affected in the first year. One would therefore expect a marked discrepancy between the number of villages twice infected and the number calculated on the assumption that the infections in two years are independent events. Of course when a very large proportion of the villages is infected in both years, the possibility of a discrepancy is diminished. For instance, suppose in a group of 100 villages, 90 are infected in one year and 90 again in the second, we should expect as a matter of chance that 81 would be infected in both years and on the recrudescence hypothesis we could not have many more than 90 affected twice. Such a discrepancy as this, when the numbers are small, would be consistent with a high value of P. While these considerations must be well weighed, it seems to me that sufficient margin exists in the groups of villages and sequences of epidemics analysed in the table to have afforded scope for the numbers of twice affected villages to exceed the calculated numbers more definitely than was actually the case. To make this clear Table XXX was prepared.

This table shows the percentage of villages in each group affected in each epidemic, which did not return cases in the previous epidemic. This shows how large a proportion of villages affected in any one year had not returned cases in the previous year (vide supra p. 363).

It therefore seems clear that the agreement between the chance distribution and the actual one is closer than we should expect were the recrudescence hypothesis actually to express an epidemiological truth.

On the other hand, if we adopt the hypothesis of importation examined in the first part of this report, we should not expect to find an absolute agreement between chance and observation, we should still expect the fact of previous infection to have some importance, for the following reasons.

What the factors may be which determine importation has not yet been rendered precise. That habitual lines of travel, *e.g.* railways, are influential is suggested by many facts, especially the maps published by Nathan in his report of the 1896 outbreak and an analysis of the plague history of Amritsar villages within two miles of a railroad which I shall publish in a subsequent report. But, in any case, it may be regarded as certain that the influences of whatever nature tend to act in the same direction for fairly long periods of time, that if any village is favourably situated for importation in 1906 it is likely to be favourably situated again in 1907. Hence, we should expect to find that villages which have been infected once are on the whole more likely to be infected in the following epidemic than villages taken at random. But since these circumstances are external to the village, and not part and parcel of the infection itself, they *may* be changed. A virulent outbreak in one year may deter visitors from approaching it in the following plague season, sanitary measures may be enacted and enforced, these and a thousand other circumstances dependent on the mutability of human actions might tend to weaken or even reverse the presumption created by a first infection. The conclusion is that while on the recrudescence theory we should expect to find a strong predisposition to reinfection in the case of villages once attacked, on the theory of reimportation, the influence of a first infection should be slighter and variable. The statistical evidence is, I think, more consistent with the second alternative.

In view of the smallness of the experience—from a statistical point of view—and the non-uniformity of the results, I am not justified in asserting that the evidence here adduced disproves the recrudescence theory. It does, however, somewhat strengthen the case against it. When this evidence is combined with that advanced in the first part of the present report, the case becomes, in my opinion, a rather strong one.

The broad conclusions which may be drawn from the present analysis are, I think, the following:—

(1) In none of the three districts can the total distribution of villages into classes showing no, one, two, etc. infections possibly be regarded as a chance event.

(2) In Rohtak, grouping villages of approximately the same size together and considering these groups separately, markedly diminishes the non-random character of the distribution. The agreement between the numbers in each group which were 0, 1, 2, 3 or 4 times affected and the numbers calculated on the assumption that the distribution was a chance one is in every case fair and in some excellent. This agreement is exaggerated by the relatively small numbers of villages in the groups and paucity of epidemics in comparison with the other districts, but when allowance is made for these circumstances, it is still evident that Rohtak yields better agreements than the other districts, in particular much better agreements than in the Amritsar groups.

(3) The Mozuffarnagar groups also show marked improvement as compared with the total for that district, an improvement not accounted for by the smaller size of the groups as compared with the total. They do not yield such uniformly good results as Rohtak but some are so excellent as to warrant one in concluding that size of villages in

Mozuffarnagar as in Rohtak is much the most important cause of the discrepancy found in the table for the whole district.

(4) Grouping in Amritsar has improved the agreement sufficiently to warrant the assertion that here also size of village has been of importance. The results, however, are not generally good and cannot, by allowing for size and number of classes, be sufficiently improved to justify us in regarding size of village as having played so decisive a part as in the other districts.

(5) Nothing in the statistical evidence affords a satisfactory explanation of this difference which may, however, profitably be made the object of further statistical inquiry.

(6) Differences in the numbers of villages within each group partly account for the differences in goodness of agreement within each set of groups, but not entirely.

(7) There is not sufficient statistical evidence that relative variations in size within the groups account for this divergence.

(8) There is no good reason to think that the fact of a village having been infected in one epidemic renders it more likely to be infected in the following epidemic or less likely to be so infected than any other village.

It may perhaps seem to the reader that the analysis here presented is not fine enough to do justice to the valuable material. While I fully recognise that the present data in all probability constitute the most valuable and complete statistical materials which have ever been collected for the study of plague, still, possible sources of error which have been pointed out to me, especially by Major Lamb, dispose me to think that an ostensibly more refined analysis might be misleading. The conclusions herein presented may not be altogether without interest and value.

TABLE I.

To show the increase in percentage of infected villages as the population increases.

[Cf. the Tables LIV—LIX *supra*.]

Mean population of Groups	Percentage never infected	Percentage 1 infection	Percentage 2 infections	Percentage 3 infections	Percentage 4 or more infections
Rohtak.					
92	80·00	20·00	0	0	0
257	51·22	31·71	17·07	0	0
352	50·00	33·33	14·67	0	0
603	30·65	32·26	32·26	4·84	0
784	20·93	39·53	34·88	4·65	0
1059	15·25	33·90	45·76	1·69	3·39
1465	6·82	25·00	61·36	6·82	0
2373	5·13	15·38	64·10	15·38	0
Mozuffarnagar.					
404	34·76	45·12	17·68	1·22	1·22
606	25·53	45·39	23·40	5·67	0
917	21·58	28·06	38·13	10·79	1·44
1247	9·76	23·17	45·12	18·29	3·66
1746	6·94	23·61	40·28	23·61	5·56
4137	4·49	15·73	33·70	29·21	16·85
Amritsar.					
175	37·78	24·44	28·89	4·44	4·44
276	24·53	30·19	18·87	20·75	5·66
308	21·88	33·13	23·75	15·00	6·25
708	7·44	20·51	22·05	29·49	20·51
1257	1·96	7·19	11·76	27·45	51·63
3837	0·78	2·33	4·65	18·60	73·64

TABLE II.

To show the difference between the results obtained by the direct method of calculation used in the other tables and the values obtained when a mean value for the probability of infection is used.

Amritsar District.

Number of epidemics	Villages infected (calculated numbers)	
	Direct method	Mean method
0	36	48·5
1	182	195·8
2	351	329·1
3	323	295·1
4	143	148·8
5	26	40
6	1	4·5

TABLE III.

Numbers of villages affected in various combinations of epidemics compared with the expected numbers.

Amritsar District.

Times affected	Actual number of villages	Calculated number of villages	Difference	Square of the difference	Square of the difference divided by the calculated number
0	155	36	+119	14161	393·36
1	183	182	+1	1	·01
2	211	351	−140	19600	55·84
3	230	323	−93	8649	26·78
4	169	143	+26	676	4·73
5	93	26	+67	4489	172·65
6	21	1	+20	400	400
	1062	1062			$1053·37=\chi^2$

P corresponding to $\chi^2=1053·37$ is too small to be tabled in Elderton's Table. Calculation from the Subsidiary Table shows P to be much less than one in a billion. In other words, if the distribution is really a chance one, we should get so bad an agreement on the average less than once in a billion trials. The distribution can hardly, therefore, be regarded as a chance one.

TABLE III *A*.

Showing the details for various years.

Amritsar District.

Number of villages affected in various epidemics and combinations of epidemics together with the calculated probable numbers.

	Actual number	Probable number
1901	1	2·2
1902	34	32·7
1903	26	25·9
1904	60	61·1
1905	3	12·6
1906	59	47·4
	183	181·9
1901 & 2	1	2·0
1901 & 3	1	1·6
1901 & 4	2	3·8
1901 & 5	1	0·8
1901 & 6	1	2·9
1902 & 3	15	23·6
1902 & 4	30	55·6
1902 & 5	6	11·5
1902 & 6	22	43·1
1903 & 4	23	44·1
1903 & 5	9	9·1
1903 & 6	13	34·2
1904 & 5	16	21·5
1904 & 6	65	80·7
1905 & 6	6	16·6
	211	351·1
1901 & 2 & 3	1	1·5
1901 & 2 & 4	0	3·5
1901 & 2 & 5	0	0·7
1901 & 2 & 6	0	2·7
1901 & 3 & 6	3	2·1
1901 & 4 & 6	2	5·0
1901 & 5 & 6	0	1·0
1901 & 3 & 5	0	0·6
1901 & 3 & 4	0	2·7
1901 & 4 & 5	0	1·3
1902 & 3 & 6	14	31·1
1902 & 3 & 4	33	40·1
1902 & 3 & 5	4	8·3
1902 & 4 & 5	10	19·5
1902 & 4 & 6	79	73·4
1902 & 5 & 6	4	15·1
1903 & 4 & 5	5	15·5
1903 & 4 & 6	44	58·2
1903 & 5 & 6	6	12·0
1904 & 5 & 6	25	28·3
	230	322·6
1901 & 2 & 3 & 4	1	2·5
1901 & 3 & 4 & 5	1	1·0
1901 & 2 & 4 & 5	0	1·2
1901 & 2 & 3 & 5	0	0·5
1901 & 2 & 3 & 6	1	1·9
1901 & 2 & 4 & 6	2	4·5
1901 & 2 & 5 & 6	0	0·9
1901 & 3 & 5 & 6	0	0·7
1903 & 4 & 5 & 6	23	20·4
1903 & 2 & 5 & 6	7	10·9
1901 & 3 & 4 & 6	4	3·6
1902 & 4 & 5 & 6	30	25·8
1901 & 4 & 5 & 6	1	1·7
1902 & 3 & 4 & 6	82	52·9
1902 & 3 & 4 & 5	18	14·1
	170 (169)*	142·6
1901 & 2 & 3 & 4 & 5	2	0·9
1901 & 2 & 3 & 4 & 6	12	3·3
1901 & 3 & 4 & 5 & 6	1	1·3
1901 & 2 & 4 & 5 & 6	2	1·6
1902 & 3 & 4 & 5 & 6	79	18·6
1901 & 2 & 3 & 5 & 6	0	0·7
	96 (93)*	26·4
1901&2&3&4&5&6	21	1·2
Never affected	155	36·0
Totals	1066 (1062)	1061·8

* The figures in brackets are those which appear in the MS. summary handed me and I used them for calculation. They do not agree exactly with the returns in the data papers, but the difference is quite unimportant.

TABLE IV.

MOZUFFARNAGAR DISTRICT.

Numbers of villages affected in various combinations of epidemics compared with the expected numbers.

Times affected	Actual number	Calculated number	Difference	Square of the difference	Square of the difference divided by the calculated number
0	334	209·60	+124·4	15475·36	73·832
1	301	461·268	−160·268	256858·3182	556·853
2	226	254·486	− 28·486	811·4522	3·189
3	86	44·866	+ 41·134	1692·00596	37·712
4	25 } 26	2·734 } 2·779	+ 23·221	539·2148	194·032
5	1	·045			
	973	972·999			865·61880

P less than 1 in a billion.

TABLE V.

ROHTAK DISTRICT.

Numbers of villages affected in various combinations of epidemics compared with the expected numbers.

Times affected	Actual number	Calculated number	Difference	Square of the difference	Square of the difference divided by the calculated number
0	145	89·26	+55·74	3106·9476	34·808
1	138	225·00	−87·00	7569·0	33·640
2	172	159·22	+12·78	163·3284	1·026
3	37	24·56	+12·44	154·7536	6·301
4	7	0·98	+ 6·02	36·2404	36·980
	499	499·02			112·755

P less than 1 in a billion.

TABLE VI.

AMRITSAR VILLAGES OF POPULATION BETWEEN 150 AND 200.

Comparison of actual and probable numbers affected.

Number of attacks	Actual numbers	Probable numbers
0	17	11·82
1	11	18·50
2	13	11·10
3	2	3·15
4	1 }	0·46
5	1	
	45	45·03

$P = \cdot 156$.

Mean Population 175, Standard Deviation 13·94, Coefficient of Variation 7·97.

TABLE VII.

Amritsar Villages of Population between 250 and 300.

Comparison of actual and expected number of attacks.

[One village only was affected in the first year of plague so that for convenience of calculation this year has been regarded as free.]

Number of attacks	Actual number of villages affected	Expected number	
0	13	8	
1	16	19·17	
2	10	17·21	
3	11	7·16	
4	3	1·36	1·456
5	0	·096	
	53	52·996	

$P = \cdot 035$.

Mean Population 276, Standard Deviation 14·16, Coefficient of Variation 5·13.

TABLE VIII.

Amritsar Villages of Population between 200 and 400.

Comparison of actual and calculated numbers attacked in various epidemics.

Number of epidemics	Actual number		Expected number	
0	35		23·65	
1	53		58·29	
2	38		52·18	
3	24		21·40	
4	6	10	3·89	4·08
5	4		·18	
6	0		·01	
	160		159·6	

$P = \cdot 001$.

Mean Population 308, Standard Deviation 62·94, Coefficient of Variation 20·41.

TABLE IX.

Amritsar Villages of Population between 400 and 1000.

Comparison of expected attacks with actual attacks.

Number of epidemics	Observed		Expected	
0	29		11·9	
1	80		64·61	
2	86		130·06	
3	115		121·49	
4	63		52·77	
5	16	17	8·78	9·01
6	1		0·23	
	390		389·84	

P less than ·0000001.

Mean Population 708, Standard Deviation 177·97, Coefficient of Variation 25·13.

TABLE X.

AMRITSAR VILLAGES OF POPULATION BETWEEN 1050 AND 1600.

Comparison of numbers actually affected with expectation.

Number of epidemics	Actual number affected		Expected number	
0	3	14	0·32	4·7
1	11		4·38	
2	18		21·64	
3	42		49·27	
4	46		52·59	
5	27		22·56	
6	6		2·19	
	153		152·95	

P = ·000031.

Mean Population 1257, Standard Deviation 172·86, Coefficient of Variation 13·75.

TABLE XI.

AMRITSAR VILLAGES OF POPULATION GREATER THAN 1600.

Comparison of numbers affected in various epidemics with the calculated numbers.

Number of epidemics	Actually affected		Expected number	
0	1	10	0·015	5·425
1	3		0·15	
2	6		5·26	
3	24		25·29	
4	42		53·29	
5	44		39·15	
6	9		5·92	
	129		129·08	

P = ·07.

Mean Population 3837, Standard Deviation 14065·42, Coefficient of Variation 366·61.

[This group includes Amritsar town.]

TABLE XII.

MOZUFFARNAGAR VILLAGES 350—500.

Comparison of actual numbers affected in various epidemics with expected numbers.

Number of attacks	Actual number		Expected number	
0	57		50·87	
1	74		82·90	
2	29		27·63	
3	2	4	2·54	2·6
4	2		0·06	
	164		164·0	

P = ·48.

Mean Population 404, Standard Deviation 58·75, Coefficient of Variation 14·54.

TABLE XIII.

MOZUFFARNAGAR VILLAGES 500—750.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of times affected	Actual number	Expected number
0	36	28·11
1	64	76·05
2	33	32·75
3	8	3·82
4	0	0·18
5	0	0·003
	141	140·91

$P = \cdot 11$.

Mean Population 606, Standard Deviation 64·57, Coefficient of Variation 10·65.

TABLE XIV.

MOZUFFARNAGAR VILLAGES BETWEEN 750 AND 1100.

Comparison of actual numbers of cases with expectation.

Number of attacks	Actual number of villages	Expected number	
0	30	15·12	
1	39	62·74	
2	53	48·97	
3	15	11·43	
4	2	0·81	·82
5	0	0·01	
	139	139·08	

$P = \cdot 000007$.

Mean Population 917, Standard Deviation 100·61, Coefficient of Variation 10·98.

TABLE XV.

MOZUFFARNAGAR VILLAGES BETWEEN 1100 AND 1450.

Comparison of numbers actually affected in different epidemics with expected numbers.

Number of times affected	Actual number affected	Expected number	
0	8	3·02	
1	19	25·12	
2	37	38·51	
3	15	13·79	
4	3	1·61	1·66
5	0	0·05	
	82	82·1	

$P = \cdot 03$.

Mean Population 1247, Standard Deviation 100·21, Coefficient of Variation 7·73.

TABLE XVI.

MOZUFFARNAGAR VILLAGES BETWEEN 1400 AND 2200.

Comparison of numbers actually affected in various epidemics with the expected numbers.

Number of epidemics	Actual number	Expected number
0	5	2·19
1	17	17·78
2	29	34·14
3	17	15·71
4	4	2·12
5	0	0·07
	72	72·01

$P = \cdot 28$.

Mean Population 1746, Standard Deviation 188·85, Coefficient of Variation 10·81.

TABLE XVII.

MOZUFFARNAGAR VILLAGES OVER 2000.

Comparison of numbers actually affected in various epidemics with calculated numbers.

Number of epidemics	Number actually affected	Calculated number
0	4	0·94
1	14	8·39
2	30	36·56
3	26	30·47
4	14	12·08
5	1	0·57
	89	89·01

$P = \cdot 0064$.

Mean Population 4137, Standard Deviation 3146·29, Coefficient of Variation 76·05.

[Includes villages of population 11,563, 19,304, 23,444.]

TABLE XVIII.

ROHTAK VILLAGES 1—200.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Observed number	Expected number
0	40	40·5
1	10	9·0
2	0	0·5
	50	50·0

$P = \cdot 76$.

Mean Population 92, Standard Deviation 68·96, Coefficient of Variation 74·81.

TABLE XIX.

ROHTAK VILLAGES 200—300.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Observed number	Expected number
0	21	18·53
1	13	18·22
2	7	3·98
3	0	0·27
4	0	0·00
	41	41·00

$P = \cdot 36$.

Mean Population 257, Standard Deviation 27·81, Coefficient of Variation 10·83.

TABLE XX.

ROHTAK VILLAGES BETWEEN 300 AND 400.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Actual number	Expected number
0	24	22·44
1	16	20·47
2	8	4·75
3	0	0·34
4	0	0·05
	48	48·05

$P = \cdot 30$.

Mean Population 352, Standard Deviation 30·34, Coefficient of variation 8·62.

TABLE XXI.

ROHTAK VILLAGES 500—700.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Observed number	Expected number
0	19	14·33
1	20	28·69
2	20	16·69
3	3	2·27
4	0	0·03
	62	62·01

$P = \cdot 28$.

Mean Population 603, Standard Deviation 57·08, Coefficient of Variation 9·46.

TABLE XXII.

ROHTAK VILLAGES 700—900.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Observed number	Expected number
0	9	7·40
1	17	19·76
2	15	14·29
3	2	1·56
	43	43·01

$P = {\cdot}82$.

Mean Population 784·23, Standard Deviation 57·36, Coefficient of Variation 7·31.

TABLE XXIII.

ROHTAK VILLAGES 950—1200.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Observed number		Expected number	
0	9		5·35	
1	20		25·67	
2	27		24·69	
3	1	3	3·21	3·29
4	2		0·08	
	59		59·0	

$P = {\cdot}26$.

Mean Population 1059, Standard Deviation 87·18, Coefficient of Variation 8·24.

TABLE XXIV.

ROHTAK VILLAGES POPULATION 1400—1700.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Actual number	Expected number
0	3	2·0
1	11	14·12
2	27	23·89
3	3	3·86
4	0	0·13
	44	44·00

$P = {\cdot}75$.

Mean Population 1465, Standard Deviation 130·92, Coefficient of Variation 8·94.

TABLE XXV.

ROHTAK 2000—2950.

Comparison of numbers actually affected in various epidemics with expected numbers.

Number of epidemics	Actual number	Expected number
0	2	0·80
1	6	8·78
2	25	23·40
3	6	5·67
4	0	0·36
	39	39·01

$P = \cdot 53.$

Mean Population 2373, Standard Deviation 230·89, Coefficient of Variation 9·73.

TABLE XXVI.

Relation between Goodness of Fit and Variation in Size.

No. in Group	Average Population	Coefficient of Variation	P
		ROHTAK.	
*50	92	68·96	·76
41	257	10·83	·36
48	352	8·62	·30
62	603	9·46	·28
43	784	7·31	·82
59	1059	8·24	·26
44	1465	8·94	·75
39	2373	9·73	·53
		MOZUFFARNAGAR.	
164	404	14·54	·48
141	606	10·65	·11
139	917	10·98	·000
82	1247	7·73	·03
72	1746	10·81	·28
*89	4137	76·05	·006
		AMRITSAR.	
45	175	7·97	·156
53	276	5·13	·035
160	308	20·41	·001
*390	708	25·13	·000
153	1257	13·75	·000
*129	3837	366·61	·07

TABLE XXVII.

Showing the influence of the non-infected villages on the Goodness of Fit.

No. of villages in the group	Percentage of total number in the group which should not be infected	Actual difference between calculated and observed number	Percentage contribution of this difference to the value of χ^2	Fit
		ROHTAK.		
39	2·05	+ 1·2	56·80	·53
62	23·01	+ 4·68	30·02	·28
48	46·71	+ 1·56	2·93	·30
43	17·21	+ 1·60	38·74	·82
41	45·19	+ 2·47	7·53	·36
44	4·55	+ 1·0	26·10	·75
59	9·07	+ 3·65	62·50	·26
50	81·00	− ·5	1·00	·76
		MOZUFFARNAGAR.		
82	3·68	+ 4·98	74·99	·03
139	10·88	+14·88	54·71	·00
72	3·04	+ 2·91	57·63	·28
164	31·02	+ 6·13	29·36	·48
141	19·94	+ 7·89	24·93	·11
89	1·06	+ 3·06	61·58	·01
		AMRITSAR.		
153	3·07	+ 9·3	64·76	·00
390	3·05	+17·1	46·73	·00
53	15·09	+ 5·0	30·14	·04
45	26·27	+ 5·18	33·94	·16
160	14·78	+11·35	29·15	·00

TABLE XXVIII.

To show the influence of Size and Grouping on the Goodness of Fit (P).

Values of *P* which result when the groups are reduced to a total of 44 villages in each case and only 5 classes are used.

ROHTAK.		AMRITSAR.		MOZUFFARNAGAR.	
Group	Goodness of Fit (*P*)	Group	Goodness of Fit (*P*)	Group	Goodness of Fit (*P*)
200—300	·32	150—200	·16	500—750	·60
300—400	·34	250—300	·07	750—1100	·08
500—700	·46	200—400	·28	1100—1400	·21
1400—1700	·75	400—1000	·24	1400—2200	·44
2000—2950	·47	1050—1600	·15	Greater than 2000	·10
		Greater than 1600	·57		
Mean	·47	Mean	·25	Mean	·29*

* Two Rohtak and one Mozuffarnagar Groups contain four classes only, reduced to a total of 44, they give :

R. 700—900 ·82 R. 950—1200 ·40 M. 350—500 ·87

TABLE XXIX.

Amritsar villages tested for recurrences in successive years of plague.

1. GROUPS USED.

Population limits	No. of villages in the group
1200—1400	46
1000—1200	67
800—1000	97
500—700	166
400—500	97

2. RESULTS ARRANGED IN GROUPS.

	1903 and 1904		1904 and 1905		1905 and 1906		1906 and 1907	
Group	No. affected in both years	Expected no. affected in both years	No. affected in both years	Expected no. affected in both years	No. affected in both years	Expected no. affected in both years	No. affected in both years	Expected no. affected in both years
1200—1400	25	23·65	27	27·83	17	14·78	10	12·93
1000—1200	26	25·03	33	30·85	25	24·52	23	22·21
800—1000	32	28·93	41	39·36	31	26·53	28	23·42
500—700	39	30·27	45	42·38	20	17·71	20	12·99
400—500	16	14·65	15	16·44	16	15·88	13	14·14
Goodness of Fit (P)	**·54**		**·91**		**·84**		**·25**	

3. TOTALS ARRANGED IN YEARS.

Years	No. affected in both years	Expected no. affected in both years	
1903—4	138	122·53	
1904—5	161	156·86	
1905—6	109	99·42	$P=$**·29**
1906—7	94	85·69	

TABLE XXX.

Percentages of villages affected in any one year which did not report cases in the previous year.

Population limits	Infected in 1904 but not 1903 (percentage of all villages infected in 1904)	1905 but not 1904	1906 but not 1905	1907 but not 1906
1200—1400	18·8	32·5	0	71·4
1000—1200	30·8	37·7	9·7	52·1
800—1000	30·4	49·4	3·1	60·6
500—700	41·8	57·1	28·6	74·0
400—500	41·4	72·7	35·7	73·5

PART III. GENERAL SUMMARY OF CONCLUSIONS.

(1) While there is an appreciable number of villages in which the interval elapsing between the last death in one epidemic and the first death in the next following epidemic is so short that it is unnecessary to postulate a re-introduction of the disease, in the great majority the interval of freedom is so long that a re-importation of the infective agent is more likely to be the cause of the outbreak than recrudescence.

(2) The villages in which the infection has been, or may have been, carried over from one epidemic to the next are generally of large size and, with the exception of certain large towns, vary from year to year.

(3) A study of the later plague history of villages infected in the milder epidemics and of villages infected at the ends of epidemics confirms the conclusions stated in (1).

(4) A study of the distribution of infected villages in maps showing the position of affairs month by month suggests a spreading out of the infection from various centres, although, when the infection becomes widespread, this may be difficult to trace.

(5) Paying no attention to the respective sizes of the different villages, the numbers attacked in none, one, two, etc. epidemics do not form a random, or chance, distribution.

(6) There is a direct relation between the number of epidemics in which villages have been attacked and the average population of such villages. The average population of villages which have never been attacked is very small.

(7) The chief factor in the non-random distribution mentioned in (5) has been the great variation in size of village within each district. In Amritsar some other factor has also been very influential, in Mozuffarnagar and Rohtak no agency other than discrepancies in size can be definitely shown to exist.

(8) The incidence of plague in the Amritsar district is unlike that found in the other two districts.

(9) The statistical evidence does not point to the conclusion that the fact of a village having been infected in any one epidemic renders it more liable to be infected in the next following epidemic.

(10) Certain villages appear to possess an immunity distinct from the relative immunity conferred by low average population.

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N°18

The FATALITY *of* FRACTURES *of the* LOWER EXTREMITY *and of* LOBAR PNEUMONIA. *A* STUDY *of* HOSPITAL MORTALITY RATES, 1751-1901.

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(From the Statistical Departments of the London Hospital and the Lister Institute of Preventive Medicine.)

[Read before the Royal Statistical Society, February 21, 1911, the President, the Right Hon. Lord GEORGE HAMILTON, G.C.S.I., in the Chair.]

I. *Introduction.*

A CIRCUMSTANCE which must have impressed itself upon many sociologists is the fact that our statistical history of disease is very imperfect. We have indeed, thanks to the labours of many distinguished epidemiologists and statisticians, much knowledge of the prevalence and types of infectious disease in the near and remote past, while Hirsch and others have shed light on geographical pathology. When, however, we turn to those common diseases and injuries which have always been observed to occur and will doubtless continue to recur in every age, our knowledge is seen to be extremely incomplete. For instance, we all know that the hospital treatment of fractures is now far superior to what could be obtained in the eighteenth century, but no continuous statistical record of the change has yet been published. Again, lobar pneumonia has been recognised as a clinical entity for many years; how has its case mortality varied? What is the relation between case mortality and age, sex or social rank? Such questions as these have, or may have, something more than an historical interest, since their solution might point to the importance of factors hitherto unsuspected in the course and termination of certain diseases. Even were this not to prove the case, the value of such inquiries, when prosecuted in the spirit of exact science, must be considerable to him who wishes to study the hygienic progress of our country.

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* *Biometrika*, Vol. VI. p. 377.

It is the object of these investigations, the first of which is now published, to provide comparable data obtained from one section of the community, viz., "The General Hospital Population" as observed in the London Hospital, and before detailing the results of our inquiry it is essential to consider some main peculiarities of this stringently selected class.

Two forms of selection have great influence in determining the characteristics of a "General Hospital Population." In the first place, there is an economic selection, large groups of the nation not being represented at all; in the second, there is a pathological selection, some forms of disease being over- and others under-represented from the standpoint of the actually observed prevalence of diseases in the community at large.

With respect to the first point, the following statements will, we think, be found to be substantially correct. The majority of hospital patients belong to the working class, but in the case of surgical in-patients a relatively greater admixture of members of the middle class exists than among the medical patients, owing to the increased expense of modern surgical treatment. From the point of view of our present inquiry, it is material to know whether the social position of the patients now treated differs much from that of the eighteenth century inmates. This is a question which we cannot answer exhaustively, but we are disposed to reply in the negative. In 1783, for instance, 21·6 per cent. of the total male patients in the London Hospital (1,139) were entered as "labourers," and 23·4 per cent. of the surgical patients (555) were so described. Taking the first 1,369 male patients on the surgical side in 1907, 17·5 per cent. were found to be entered as "labourers." Remembering that the word "labourer" when used without qualification now denotes only the least skilled and worst paid type of manual worker, the improvement suggested by these figures may well have been slight. Indeed it may be said that not only are the bulk of hospital patients taken from the working class, but from among the less prosperous members of that class. The existence of so high a percentage of labourers is perhaps evidence of this, but, in addition, anyone acquainted with hospitals will endorse the evidence, given before the recent Royal Commission on the Poor Laws by an experienced hospital administrator, to the effect that the line dividing the class of legal poor from that of the general hospital community is by no means distinct.

With respect to the second form of selection enumerated above, our knowledge is more definite. At the present time chronic diseases, unless their rarity makes them of special interest, are not admitted to the hospital at all freely. This selection has been practised with ever increasing stringency as the demands on hospital space on the one hand and the municipal provision for the sick poor on the other have grown. Indeed these facts have led in certain quarters to the conclusion that hospitals are not entirely satisfactory instruments for the training of the general medical practitioner. In the days before the creation of efficient parochial infirmaries such selection was less rigid, as is clear from a quite cursory examination of the eighteenth century records. There is actually some little reason to think that a precisely opposite rule was for a time followed. In this way only can we account for the high percentage of cases entered as "rheumatism" between 1750 and 1800, cases which appear seldom to have had a fatal issue, while acute pneumonia or true "peripneumony" is hardly represented at all before 1780. A plausible explanation is that in the early days of hospitals popular belief in the efficacy of institutional treatment in acute diseases was but small. There were also definite prohibitions; venereal diseases were excluded for several years. "In 1756, a list " of diseases was put up, and patients suffering from these were not " to be admitted. The 'prohibition list' included 'ulcerated cases,' " women with child, children under 7 (except for amputation or " 'cutting for stone'), patients suffering from fits, small-pox cases, " cases of itch, distempers, consumptives, asthmatical cases, infectious cases and all 'disordered in their senses.'"[1]

It may be inferred from these facts that any comparison of death-rates using a broad classification must be hopelessly misleading, and that care must be exercised in choosing materials for comparative study. These facts are further supported by a description of the records available for comparison to which we now turn.

The elaborate system of clinical notes common to the "London" and other medical schools, only extends back in our case through some thirty years. Before that time the physicians compiled case books, certain volumes of which are in existence and refer to so early a period as the third decade of the last century. These volumes are, however, of no service to the statistician since they describe but a comparatively small percentage of the total patients, the writers having naturally preferred to dwell upon cases of peculiar professional interest. The only continuous record of hospital practice is the series of admission books; these extend from 1751 down to the present time, with the exception of a period of some fifteen years at the beginning of the nineteenth century and an odd year or two more recently. These volumes record the name, age (except for the first eight or nine years), sex, occupation and disease of every patient admitted to the hospital, the length of stay in the wards and the result of treatment, whether "cured," "dead" or "relieved." With the advance of medical knowledge, the diagnosis has become more precise, but no material change has been made in the form of the entries down to the present time; we are thus in possession of an almost continuous record of nearly a century and a half's hospital experience.

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[1] *A history of the London Hospital*, p. 109, by E. W. Morris. London, 1910.

For the medical antiquary these books, with their frequent entries of diseases either extinct or no longer endemic in the east of London (*e.g.*, various agues) and their variations in nomenclature, contain much of peculiar interest. For our statistical object it is more to the point to consider what weight should be assigned to the diagnoses for comparative purposes, and how we can grapple with the selective difficulties already mentioned.

Clearly we must choose some disease or injury which (1) is common, (2) is always likely to be admitted to the hospital when it presents itself, (3) does not offer unusual difficulties in diagnosis. Quite apart from the question of selective rules, it is certain that many of the early records are of no value to us. Under the simple rubric "fever" are, for instance, a large number of entries which in all probability refer to quite disparate conditions. Indeed a considerable proportion of the diagnoses are evidently symptomatic. Even if there had been no selection, we should have been compelled to fall back upon a small field for choice. For these reasons it appeared to us that the most suitable material would be fractures of the lower extremity as an example of injury, and lobar pneumonia as a type of disease. Both these groups conform to the three conditions enumerated above, and, in addition, it may be said that the case mortality or, to use a better term, the fatality of both is appreciable even in modern times. It seemed to us that a study of this material ought to throw light on such changes as have occurred in the general conditions of hospital treatment as well as in the resistance to injury and disease of the hospital type of patient, *in so far as these can be measured by death-rates.* The first part of this paper will be devoted to a study of the statistics of (1) simple fractures of the leg, (2) simple fractures of the thigh, (3) compound fractures of the leg. Since, however, these statistics are chiefly of historical interest and do not, for reasons which will be explained later, admit of detailed analysis, we shall deal with them as briefly as possible, passing on to the second part of our work which consists of a study of the case mortality (fatality) of acute lobar pneumonia as modified by age, sex and class. This part of our inquiry is, we hope, of some little interest, since not only does our material comprise upwards of 5,000 cases, covering more than fifty years' experience, but in addition many series compiled by other writers are available for comparison with and confirmation of our results.

A. *Simple fractures of the leg.*

Table I contains the London Hospital figures from 1751-1901, grouped in decennial periods, the mean age, standard deviation and coefficient of variation being given in each case. Diagram 1 gives the fatality in the form of a graph, the separate figures for males and females from 1843 are given in Tables II and III, while separate male and female graphs from 1831 are shown in Diagram 1. It

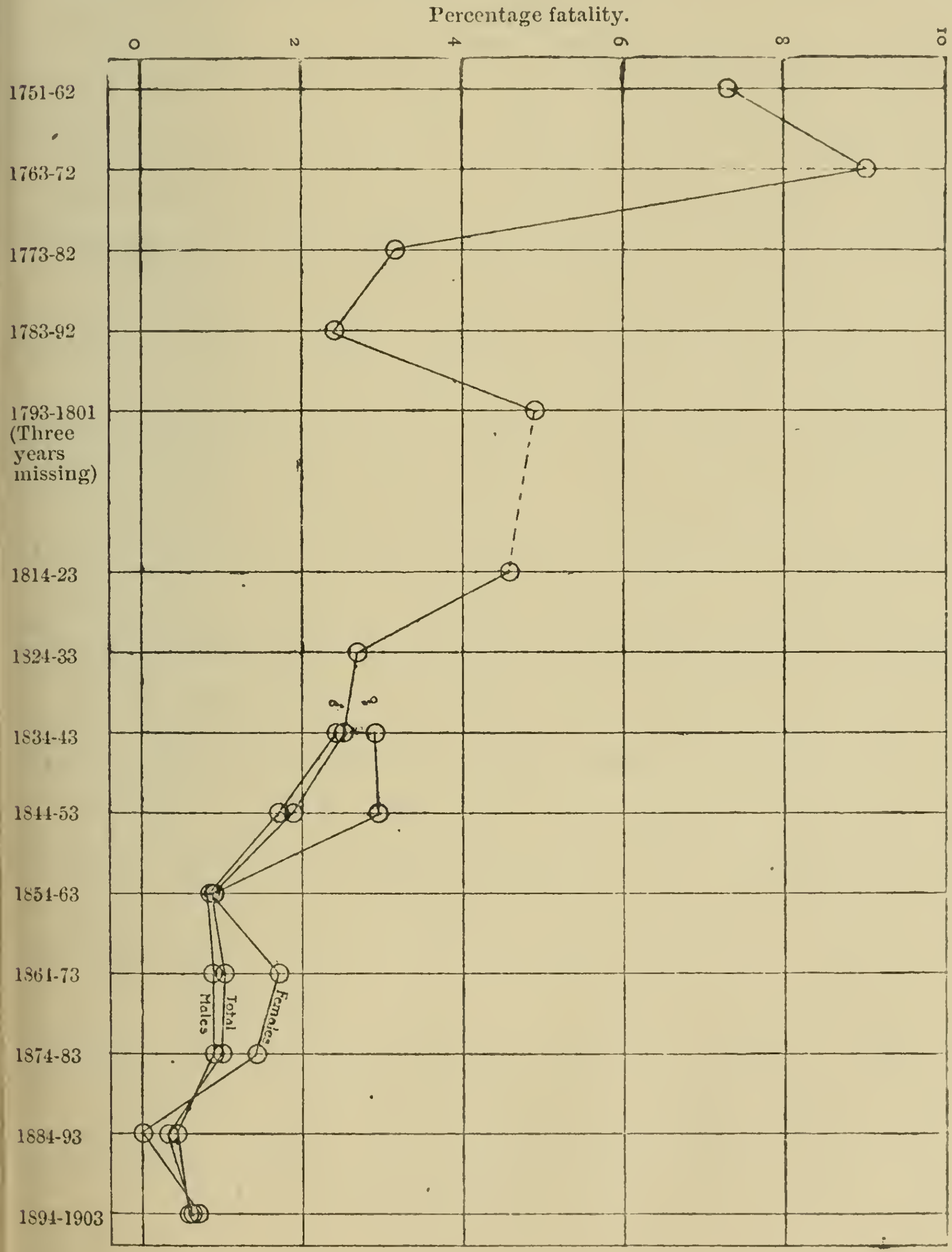

DIAGRAM 1.—*Percentage case mortality. Simple fractures of the leg.* London Hospital. In-patients. Grouped in decennial periods, 1751-1903.

should be remarked that from 1854 onwards we have not extracted the whole of the figures for each decennium, owing to the excessive labour involved; we have started with the central years of the decennium, and worked backwards and forwards. Thus the figures given for 1864-73 are based on the years 1866, 1867, 1868, 1869, 1870, and 1871. The actual omissions are shown in brackets on Table I; for the last three decennia the middle four years in each case were used. Of course, for fractures of the thigh and the other subjects considered in this paper, all the data were extracted.

Fixing our attention first on the general figures, without regard to sex, it will be seen that the fatality diminished rapidly and tolerably steadily to the decennium 1854-63; since that period it has exhibited no regular change. Two factors necessarily influence the case mortality, viz., age and sex. With regard to sex, it is clear from Tables II and III that a decided difference exists in favour of males, a difference which is still more considerable in the case of fractures of the thigh, and will be mentioned again in the next section. At present it is sufficient to remark that, in the earlier years of the hospital, female patients were not, we think, relatively more numerous than at present, so that the general diminution in fatality cannot be attributed to a change in the sex ratio. With regard to age constitution, it will be noticed that the mean age of the patients has tended to fall and, since increasing years must generally be unfavourable to a person's chance of recovery from accident, this fact may have had some influence on the fatality rate. In order to estimate the probable importance of this, we have determined, by Pearson's new method,[2] the correlation between age and liability to death after fracture. Table IV shows the groups used and the results; the probable error has been assumed to be three times that of the coefficient as determined by the product moment method in the case of normal correlation. All the values indicate an appreciable relationship, but the change in rate of fatality can hardly be attributed to this cause. Table IVA is an attempt to eliminate the age influence by the method of standard population. The mortality coefficient for each age-group (the first group was 1—5 years, the second 6—15, thence in groups of ten years) was determined for each set of data appearing in the table, then the number of deaths per 1,000 which would have occurred in a population having the age-constitution of the data for 1884-1903. It will be seen that taking a period of some thirty years as the unit instead of a decennium makes the fall smoother. At the same time, such a table as this must not have too much weight attached to it. The fatality rates are subject to large random errors, especially in the later years when the total numbers of deaths are small. It seems, however, tolerably clear that the change in fatality is a real phenomenon, not due to incomparability of the records.

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[2] *Biometrika*, vii, 96.

Changes in the rate of mortality do not, of course, completely represent the improvements which may have been effected in hospital treatment. There is, indeed, reason to think that the percentage of serviceable limbs which follow the healing of a fracture is higher in modern times; we have not, unfortunately, any statistical evidence bearing on the matter, our data only being concerned with deaths. We do not know how many of the patients returned as "cured" in the old times actually left the hospital lame for life.

A source of error which has probably occurred to the reader may be mentioned at this point. The majority of the fractures recorded in our series are the result of direct or indirect violence; in many cases they are the results of street accidents; in others of falls from the rigging of ships, and so on. Now it might well happen that a person admitted to the hospital with a broken leg, as the only objective sign of injury, was in reality suffering from "shock," or perhaps some grave internal lesion. Supposing death to follow in such a case it would not be in any real sense a result of the broken leg, and the frequency of such deaths would throw no light upon changes in hospital treatment; the inclusions of these cases would indeed be a serious source of error in our investigations. To minimise this we have omitted all cases in which death took place within three days of admission to the wards. This plan, of course, reduces the total mortality, but we feel confident some such method must be adopted, and have followed the same course in the case of compound fractures of the leg and simple fractures of the thigh. Even by this process we cannot have excluded the whole of the instances in which death was not really due to the fracture as a primary cause: for instance, a few undetected cases of sarcoma and some of shock or internal injury which survived longer than three days. In view of the large numbers involved it is to be hoped that such sources of error are not of importance.[3] An unavoidable source of error is mistake in diagnosis; perhaps in this particular case the number of such errors is not very large.

One further point of interest in connection with simple fractures of the leg has been examined. In the later years the fractures are classified in accordance with whether one or both bones were broken, and it seemed to us that the age distributions were markedly different in the cases of separate bones; tabulation proved this to be the fact, as will be seen in Table V. For the purposes of this comparison no cases of Pott's fracture[4] were included. We thought it might be of interest to fit a frequency curve to the cases of fractured fibula; Table VI contains the results, from which it will be seen that a good fit was not obtained. We are unable to say whether this is due to heterogeneity of the material or inaccuracy of the age records. It is not very easy to say why this marked difference in mean age should be found; had we included Pott's fracture the cause would have been plain, since the form of accident which generally produces this kind of injury is more likely to occur in later life. As it is, however, we can only refer the distinction to the fact that the superior elasticity of youthful bones would perhaps be a greater safeguard to the fibula, a slender rod fixed at the extremities and bearing little direct weight, than to the massive tibia.

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[3] It is also to be noted that the case mortality rates in the later years depend on small absolute numbers of deaths, and are subject to large random errors.

Finally, we determined whether the age distribution for all fractures of the leg was markedly different from that of the population at large. To ascertain this, we extracted the data for the population at ages from the census of 1901, Metropolitan Borough of Stepney, which includes the district in which the accidents likely to be treated in the London Hospital would occur. We then tabulated the actual ages of the patients admitted with fractures in 1897–1900, together with the probable age distribution of the same number of cases on the ground of the census figures (Table VII); as was to be expected, the actual figures show an immensely greater incidence of fractures upon adults and a less incidence upon children than calculated on the basis of a random distribution.

The general conclusions which may be drawn from this analysis are, we think, the following:—

(1) The fatality of fractures diminished very greatly and fairly steadily during the first century of the hospital's experience.

(2) In the last fifty years the rate of fatality has not fallen regularly and has possibly reached the irreducible minimum.

(3) There is a slight sexual difference in fatality.

(4) The mean age of fractures of the tibia is appreciably lower than the mean age of fractures of the fibula.

(5) The maximum frequency occurs between the 35th and 55th years in males.

B. *Fractures of the thigh.*

These fractures have been tabulated in the same way as those examined in the last section except that all the data have been entered, even for the later years (Tables VIII—X). An examination of Table VIII fails to reveal any continuous change, the figures being most irregular. Tables VIII and IX show a striking difference between male and female rates of fatality, but this cannot be altogether sexual since the mean age of the female cases is much higher than that of the male patients and the correlation between age and liability to death (Table XI) is appreciable. We publish this set of tables for the sake of completeness and to avoid the imputation of having condemned data without giving other workers the opportunity of forming an independent judgment. In our opinion, their irregularity is so great that no sound argument can be based upon them with regard to changes in fatality. It is perhaps safe to say that they afford some evidence of a higher rate of case mortality among female patients, so far confirming the results of Section A, but beyond this we cannot go. It should be remembered that fracture of the thigh is a considerably more serious injury than fracture of the leg bones, when produced in adults.

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[4] A fracture of the lower end of the fibula, generally due to indirect violence, as in slipping over the kerb.

C. *Compound fractures of the leg.*

Since, by definition, a compound fracture supposes the existence of an open wound, it was to be expected that our returns would show a diminution in mortality, an improvement becoming more marked as we approached the present time; Table XII bears out this expectation, so far as it goes. The grouping is not for equal intervals of time, but so chosen as to give comparably large numbers. Here again, we are unable to attach very much importance to the results, owing to numerous sources of error. In the first place, the numbers are too small to allow of separating males from females; in the second, the injuries covered by the term "compound fracture" are extremely diverse in severity; in the last, amputation in very serious cases is much more likely to have been resorted to in modern times than in pre-anaesthetic days, and these cases do not figure in our returns. Nevertheless, even allowing for all these sources of error, it is not unfair to conclude that a considerable saving of life has been brought about.

On the whole, the reader will probably think that the positive additions to our knowledge which have resulted from this analysis of accident fatality are neither many nor important. It was, however, necessary to examine and analyse the data, since only by doing so could we arrive at an opinion as to their value, and perhaps save other statisticians a somewhat fruitless task.

PART II.—*The statistics of lobar pneumonia.*

In obtaining data for this part of our inquiry we have extracted cases in which the substantive disease was recorded as pneumonia, lobar pneumonia, or croupous pneumonia; all cases described as broncho-pneumonia, and any cases in which owing to ambiguity of description or other circumstance it could be fairly inferred that the disease really might be broncho-pneumonia, were rejected. Since pneumonia may occur as a complication of other morbid states, for instance, the so-called terminal pneumonias, these data do not include all cases which have been actually treated in the wards; they do, however, include all cases in which pneumonia was the principal disease. Our chief source of difficulty has been cases simply recorded as pneumonia; doubtless some of these were really instances of broncho-pneumonia, and should not have been included in our figures. This source of error was especially felt in dealing with the early years of the hospital, and with records of children. This probably explains the great irregularities to be noticed in our age groups 0—5 and 0—10 years, as well as those to be seen in the figures from other hospitals.

The crude results of our work are given in Table XIII. It will be seen that the total numbers of cases up to 1854 are so small that little weight can be assigned to them, and in what follows our attention will be mainly concentrated on the returns from 1854 onwards. Our first question is, whether the fatality has changed so markedly within the period under review that the data ought not to be treated as a whole. Table XIV contains the results of an inquiry directed to this point. If the entries in this table are compared with their probable errors, the conclusion at which one arrives is that, although on the basis of the numbers recorded it would not be safe absolutely to deny the possibility of some change having occurred, yet, except in age group 1—5 years, no marked alteration can be demonstrated. But, as we have just remarked, these early ages are precisely the ones for which our data are least trustworthy. Hence we are unlikely to commit a serious mistake in examining the data as a whole, as is done in Table XV.

Noteworthy features of this table are (1) the high fatality in the first age group, (2) the very low fatality for ages 6—15, (3) the rapid rise after the twenty-sixth year (see Graph 2). The probable errors of the percentages as determined by the usual method are given in the table; the process is, however, not really valid when the percentage of the character falls much below 10 per cent. In such cases the distribution of random samples is not effectively given by the normal curve.[5] One of us hopes to discuss this question at length on another occasion, since the matter is of importance in some classes of medical inquiries, but the problem need not detain us at present.

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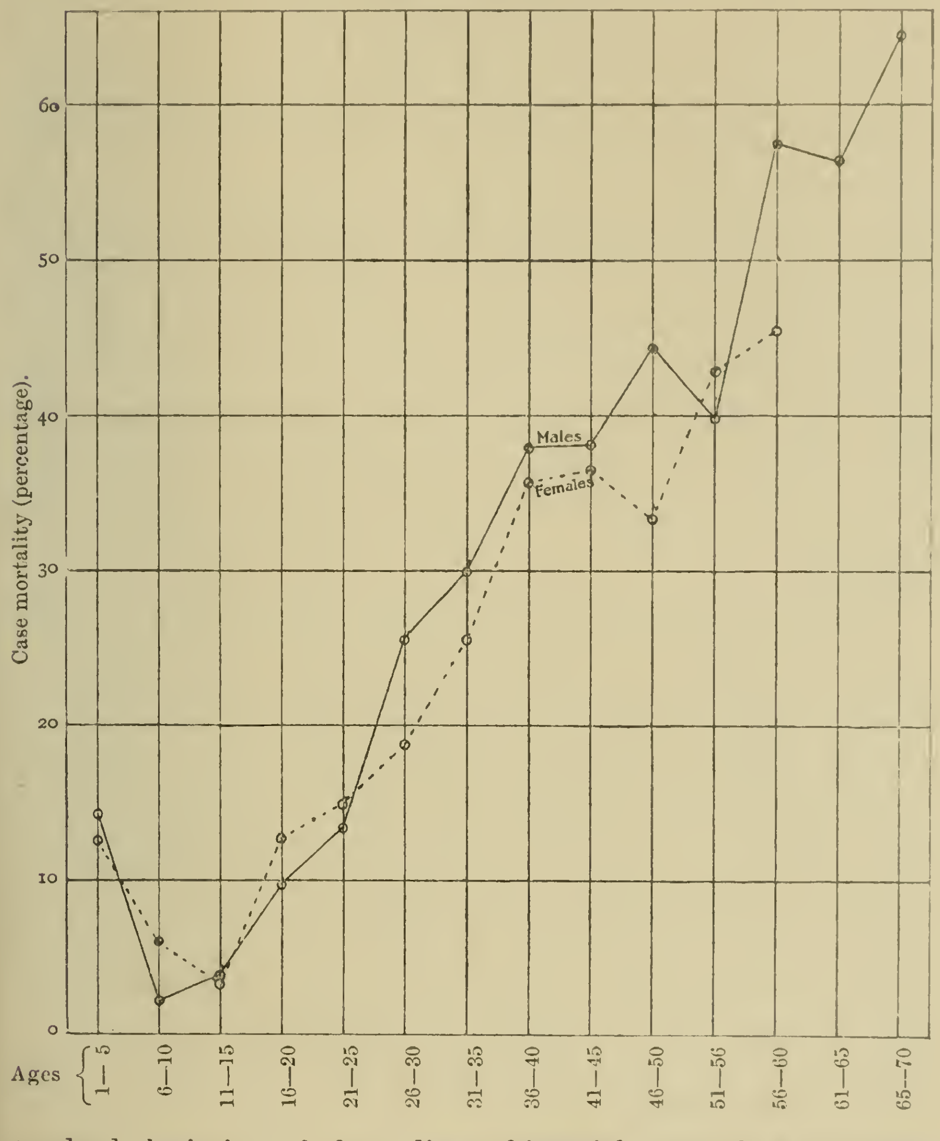
[5] Pearson, *Biometrika*, v, 172.

There is, however, another objection to the conventional method of determining the probable error when applied to data such as these which deserves serious notice. The process of deducing the standard deviation of the ordinary binomial can only be regarded as a valid test of dispersion when the chances of the events happening are strictly independent. It is assumed that in a population of n individuals p of whom possess a certain character, the chance of any individual presenting the character is p/n. Even when the events are independent, the dispersion is not always the same; an elementary illustration being the difference between the dispersions obtained on the basis of Bernoulli's scheme and the more general

DIAGRAM 2.—*Case fatality of pneumonia (lobar).*

London Hospital, 1854-1903. Total number of cases: males, 3,791; females, 1,306.



case of Poisson.[6] But if we reach a case in which the chances are not independent, the difficulty becomes much greater. If in a population of n, p suffer from an infectious disease, it cannot be said that the chance of any one person catching the disease is absolutely independent of the chance of any other person taking it. In such a case it seems absolutely necessary to deduce a new expression for the frequency of random samples. One of us has devoted some little attention to this problem, and hopes to communicate a few theoretical results later. We do not think the problem, as a question of algebra, offers any insuperable difficulties, indeed, such difficulties as have so far been encountered would probably give little trouble to a more expert analyst. The crux seems rather to be as to the objective assumptions to be made. We must suppose that the population presents various degrees of susceptibility; further, we have to consider whether the infective organism progressively changes in virulence throughout the epidemic. On both these points some statistical work has been done. We may refer to the papers of Brownlee on the course of epidemics, and to Yule's discussion of a cognate, but not identical, problem[7] in the Journal of this Society. The immediate point is, how far do these considerations invalidate the probable error test as applied to our material?

It is to be remarked that we are not dealing with the number of cases of pneumonia occurring in the population, but with the number of deaths among pneumonia patients. The difficulty of infection does not arise, since the whole "population" is infected; we might, however, suppose that the deaths are not independent, since a group of deaths might be caused by the patients all having been infected by a specially virulent strain of pneumococcus, or having all been subject to the influence of unfavourable weather conditions. There can be little doubt that climatic conditions markedly affect the prevalence of pneumonia; whether they also change the case mortality is, perhaps, not quite so certain, but still likely enough. It is also highly probable that variations in the virulence of the infective organism or its toxins occur. These considerations weaken the value of the test, but some mitigating circumstances should be noted. In our records a period of ten years is the unit of time, and the patients are drawn from a fairly wide area. Perhaps the hypothesis that the causes of the individual events are independent is not entirely misleading in such a case.

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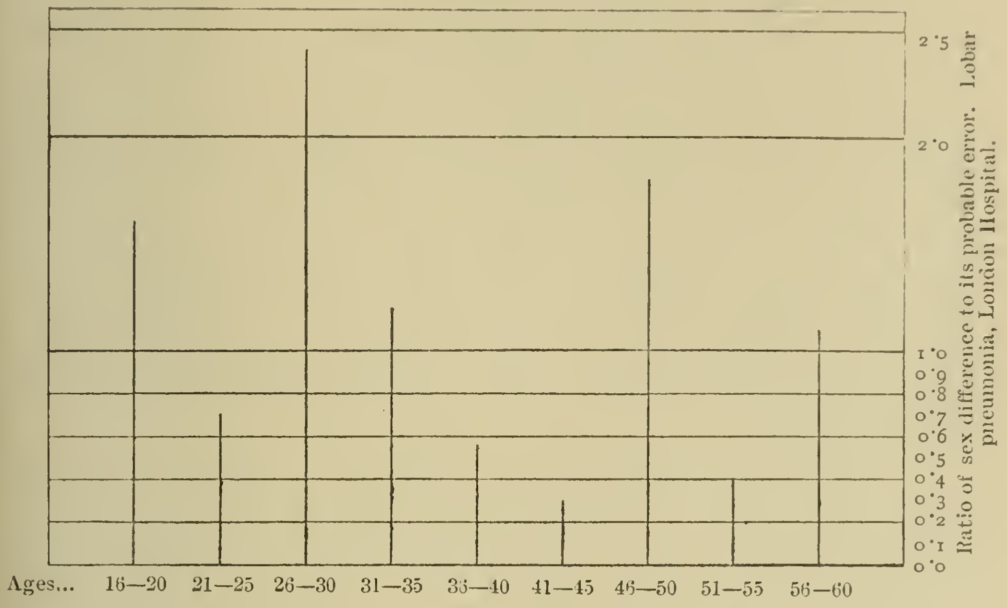
[6] For a good discussion of this, see Czuber, *Wahrscheinlichkeitsrechnung* (1903 edition), pp. 128—130.

[7] Brownlee, *Proc. Roy. Soc. Med.*, May, 1909. Yule, *Journal of the Royal Statistical Society*, lxxiii, 26.

On the whole, but with considerable hesitation, we have decided to publish the probable errors based on the ordinary calculation; we frankly recognise, however, that their interpretation is a matter of serious doubt.

The next question that arises is with regard to the difference in fatality rates of males and females. Table XVI and Diagram 3 give the necessary information on this point. The general trend of the figures rather suggests that women are somewhat more likely to be victims in early life and slightly less subject in later life than males; but an examination of the probable errors (bearing in mind the caution just given) hardly inclines one to believe that there is a real sexual difference. It would appear that sex is not a very important element in prognosis. We may remark that the actual incidence of pneumonia is said to be much heavier upon males than upon females, but we have no data which enable us to examine the problem. It is perfectly true that both our own and almost every other collection of hospital statistics show a higher proportion of male cases, but this need not be due to a greater incidence of the disease. We must remember that lobar pneumonia is an acute disease, the stress of the illness falling upon a comparatively short period. We believe it to be a fact that women of the poorer classes are specially reluctant to leave their home and children when ill, since during the period of convalescence they can still exercise some supervision over household affairs. They are more likely to remain at home if this be possible than are their

DIAGRAM 3.



husbands, who, if confined to bed, even when convalescent, can be of little use in keeping house or looking after the children.

We next have to consider how the London Hospital experience compares with that of other general hospitals, and Table XVII[8] summarises the published experience of several other institutions. The data from St. Bartholomew's Hospital appear to suggest an unusually low rate of fatality, but the numbers are rather small, and the general impression one derives from the whole of the data is that our Table XV represents quite accurately the rate of fatality in the general hospital class of patient. It is interesting to note that the data from the Johns Hopkins Hospital are derived from a population racially different from that of the other institutions; the numbers under review in this case are too sparse to permit of very definite conclusions being drawn, but, so far as they go, there seems little reason to expect a racial difference in fatality rate. In view of the fact that our material is tolerably extensive, the statistician will naturally inquire whether we have attempted to graduate the fatality percentages by any formula allowing of interpolation; this is an interesting question, which we have approached in the following way:—

The fatality of the group up to 5 years is so variable (see, *e.g.*, Table XIV), and the impression that lobar pneumonia is not a very fatal disease in young children so general among experienced clinicians, that we suspect strongly that the data for this age group are subject to a large empirical error. It is indeed well known that the differential diagnosis of lobar pneumonia in young children is not an easy matter,[9] so that we are fairly confident but little reliance ought to be placed upon these figures. We therefore confined our attention to the remainder of the experience, and attempted to graduate it. Two methods suggested themselves; the first was to fit a straight line by the method of least squares, or, what comes to the same thing, and is slightly quicker, to determine the regression line of percentage fatality upon age, weighting, of course, with the number of observations in each age class. The second was to consider the percentages as ultimate facts, and to fit a frequency curve to them by moments.

Obviously the second plan, which neglects the fact that the percentages at the end of the experience are subject to large probable errors, owing to their being based on a small absolute number of instances, can give nothing but an interpolation formula having no general validity; we thought, however, the comparison of the results might be of statistical interest. Table XVIII contains the results obtained by both processes. The straight line was determined by least squares, and also, as a check on the arithmetic, from the regression equation; portions of the straight line and curve are shown in Diagram 4. Since the constants of the straight line were obtained by using weighted points it was to be expected that the skew curve, fitted to the percentages themselves, regarded as actual frequencies, would follow the latter more closely, especially towards the end of the experience. Such is the case, but the curve so fitted, viz., on a purely arbitrary assumption, is not so far superior in its power of reproducing the trend of the observations to the straight

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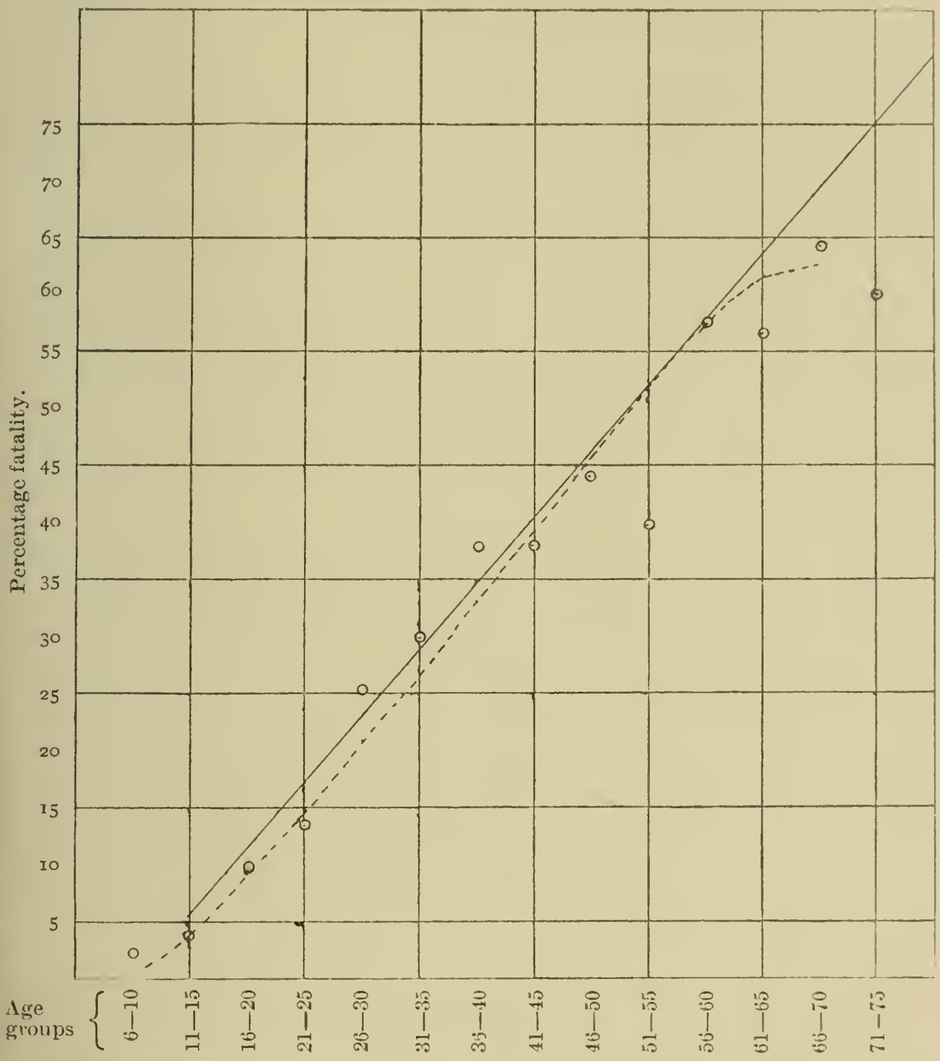
[8] Hadden, Mackenzie and Ord's data are from *St. Thomas' Hospital Reports*, 1889, Sturges and Coupland's from the 2nd edition of their work on Pneumonia, other sources appear in the table.

[9] *The natural history and relations of pneumonia*, by O. Sturges and S. Coupland. 2nd Edition. London, 1890, see esp. pp. 135—144.

DIAGRAM 4.—*Relation of age to mean fatality.*

London Hospital. Male pneumonia cases.



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* *Biometrika*, Vol. VI. p. 377.

line that it can be regarded as preferable even as an interpolation formula. When we remember the undoubted fact that the ages as given in our records are almost certainly subject to a considerable observational error, owing to ignorance or carelessness on the part of the patients, we may say that the results of our analysis suggest that, so far as mere curve fitting is concerned, fatality may quite possibly be a linear function of age. It is, of course, not improbable that a weighted parabola would represent the observations still better, but in view of the imperfections of the data just mentioned, it seems that the straight line is a close enough representation of the data, and might be used for purposes of interpolation with sufficient accuracy. Naturally such a line could not be used to extrapolate fatality rates outside the range of our observations; indeed it is well known that even with data much surpassing ours in extent and accuracy extrapolation is a dangerous process.[10] It is worth notice that the true nature of the relation between age and *severity* cannot be completely determined in this way. Our scale of severity is only the percentage of fatal cases upon total cases at each age; we have no means of determining the real variability of the whole population. The whole value of the determination of r in such a case depends on the closeness or otherwise with which the regression line fits the observations. Without some measure of severity other than percentage deaths it hardly seems possible to use a better graphic method.[11]

Lastly, we have compared the age constitution of the experience for 1899–1903, male cases, with that of the census population of the borough of Stepney, just as was done for fractures. As will be seen, there is some evidence that the greatest incidence tends to come upon the group 15—19, but there is nothing like the discrepancy observed in the case of fractures. This result is in accordance with one's expectation, since we should anticipate that the cases would tend to be evenly distributed on the whole with the exception of some increase in the years of early manhood when exposure to outdoor conditions might tend to lower the resistance of the organism towards the infective agent.

The results so far considered seem to justify the following statements: (1) The fatality of pneumonia in the hospital class of patients has not appreciably changed in the last fifty years; (2) there is no evidence of a sexual difference in fatality rate; (3) the increase of percentage fatality with age can be *roughly* represented by a straight line. The next question, and that one of much interest, is—how does hospital experience compare with that derived from private practice among other classes of the population? Is pneumonia distinctly more fatal among the poorest classes of the community than in general?

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[10] Perrin, *Biometrika*, iii, 99.

[11] For a good discussion of scales of severity see Brownlee, *Biometrika*, vi, p. 313.

Material for studying this question is not readily obtained. Indeed we know of only one published series of English cases at all comparable in respect of numbers with those just considered. These are the results of an inquiry by the Collective Investigation Committee of the British Medical Association, carried out some twenty-five years ago. The statistics of this inquiry as arranged by Sturges and Coupland[12] are given in Table XX. In the face of the comparatively small total numbers and the apparent differences between the male and female rates, it is difficult to express an opinion; we would merely remark that such figures would not, taken alone, permit one to assert that the fatality in non-hospital patients is markedly different from that shown by the hospital records; fortunately a much longer series of cases has been recently published. This material is derived from the experience of the Leipzig Krankenkasse;[13] we reproduce in Table XX the data for male obligatory members of this Krankenkasse, the experience covering a period of some ten years from 1887. The cases included are all those not diagnosed as broncho-pneumonia and may possibly comprise a few examples of the latter together with, as the editors of the official statistics suspect, some cases of tuberculous disease. It is doubtful, however, whether the data are markedly less accurate from the diagnostic standpoint than our own and, with respect to age records, it is very probable that they are more accurate than hospital figures. Since the termini of the quinquennia are not the same as were adopted in our original grouping of the London Hospital figures, the latter have been re-grouped to allow of direct comparison, and are repeated in Table XXI, which also shows the differences in rates of fatality together with their probable errors for some of the more populous groups. It will be seen that there is a literally enormous difference; in practically all the age groups the German fatality rate is little more than half that of the London Hospital patients. The question at once arose as to whether the distribution of fatality and its relation to age was materially different in this case. Table XXI and Diagram 5 show the results of a graduation. As before a weighted straight line fairly represents the data and is not markedly inferior in fit to a skew curve arrived at on the basis of the arbitrary assumption already mentioned. The change in rate of fatality with increasing age seems to follow a course similar to that observed among the London Hospital patients although the absolute values are different.

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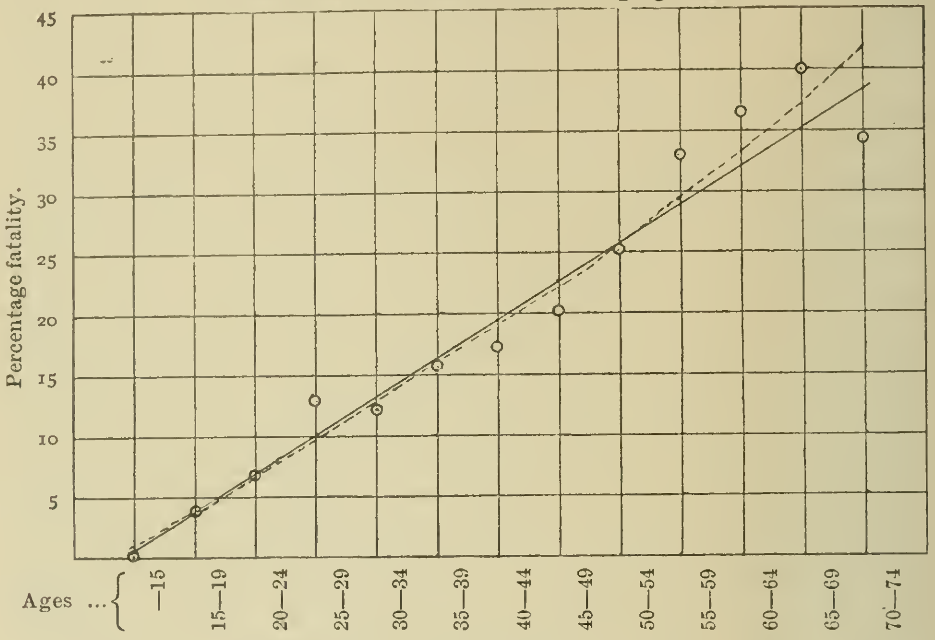
[12] *C.I.C. Reports*, Vol. II, p. 102. See also Sturges and Coupland, *op. cit.*

[13] *Krankheits u. Sterblichkeitsverhältnisse i. d. Ortskrankenkasse f. Leipzig u. Umgegend.* 4 Vols. Berlin, 1910.

DIAGRAM 5.—*Relation of age to mean fatality.*

Pneumonia. German males. Leipzig data.



We must next ask ourselves whether this great difference is due to racial or climatic elements, since there is some ground for thinking that both of these factors have some importance in the prognosis of the disease. The matter could at once be decided by reference to the statistics of a large hospital in Leipzig, but we have unfortunately not been able to find any long series of such data relating to modern times from any Continental hospital; the only data at all comparable in point of numbers which we have discovered refer to a period more than forty years ago. Fismer gives the following records for the Basel hospital:—[14]

Years.	Number of cases of pneumonia.	Deaths.	Percentage fatality.
1839–48	223	55	24·7
'49–57	197	49	24·9
'58–66	232	60	25·9
'67–71	270	49	18·0

The author attributes the diminution in the last period to the adoption of a different form of treatment, but as we cannot compare the age constitutions for the earlier periods with those of the later years, it is difficult to regard the inference as certain. On the whole, we may say that these figures do not differ materially from those of the London Hospital. In 1861 a German translation of Huss's monograph, dealing with the experience of the Seraphim Hospital at Stockholm, was published.[15] The statistical portion of this work is, in our opinion, admirably carried out, and we have collected the figures in Tables XXIII and XXIV. Although we have not been able to tabulate the information in a manner accurately comparable with the London Hospital and Leipzig data, still it is clear that the rate of fatality is much lower than that of the London Hospital, and compares not unfavourably with that of the Leipzig Krankenkasse. Were we able to suppose that the experience of German hospitals is similar, then the difference between the London and Leipzig returns would have to be attributed to racial and climatic peculiarities. Huss, however, states that between 1847 and 1857 there were 5,990 cases of pneumonia in the general hospital of Vienna, with 1,441 deaths, or a fatality of 24·13 per cent. The authority for this statement, a report issued from the Viennese hospital in 1857, is not accessible to us, but Huss is unable to suggest any satisfactory explanation of the difference. Had there been any gross fallacy in the comparison, *e.g.*, a marked difference in age constitution, we feel sure so careful a writer would have mentioned it. It would appear, therefore, that we cannot definitely say whether the hospital fatality rate in such a city as Leipzig is or is not lower than in London. If we attribute the difference between the figures from Vienna and Basel on the one hand, and Stockholm on the other, to climate, then we should probably have to say that Leipzig ought to be approximated rather to Basel and Vienna than to Stockholm. It should also be remarked that the stress of industrial conditions—the whole group of circumstances which go to make up life in a great city—render Leipzig, Vienna, and London more homogeneous from this standpoint than would be the case if we included Stockholm. We conclude, but with much hesitation, that the evidence before us is not sufficient to allow of our saying that the difference between the Leipzig and London rates should be referred to climatic or racial factors; that they cannot be due to any sudden increase in morbidity as a consequence of some local epidemic[16] is rendered probable by the length

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[14] Fismer, "Die Resultate d. Kaltwasserbehandlung b. d. ac. croup. Pneumonie." *Deutsch. Arch. f. Klin. Med.* 1873, XI, 391.

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[15] Huss, *Die Behandlung der Lungenentzündung und ihre statistisch. Verhältn. nach 16-jähr. Erfahrung aus d. Seraph-Lazarette i. Stockholm.* Übersetz. v. J. Anger, Leipzig, 1861.

[16] For a discussion of epidemics of pneumonia and the geographical distribution of the disease, see Hirsch, *Handbook of Geogr. and Hist. Pathology* Creighton's trans., vol. iii, chap. 6.

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of time under review; it seems necessary to resort to some other hypothesis. A possible cause of the discrepancy is found in the suggestion that only severe cases apply for hospital treatment. While it may be safely asserted that *any* case of pneumonia applying to a hospital will be received as an in-patient, it is possible that only the worst cases do actually apply, and that the hospital does not get a fair sample of cases of pneumonia even from the industrial class which furnishes the hospital population.

This point was investigated by Huss. He found that the fatality of pneumonia in the Stockholm Military Hospital (670 cases) was 7·31 per cent. Cases occurring among the soldiers would be seen and treated from the onset, and not submitted to the form of selection just noticed. All these patients must have been between the ages of 20—40 years; and his hospital cases gave for the same age group a fatality of about 11 per cent. On the basis of these figures it would seem that the fatality of the hospital cases is roughly eleven-sevenths of the fatality of cases treated from the onset. This is, of course, an extremely rough approximation, and probably exaggerates the difference, since the soldiers will be a physically select class. Using it as a rough check, we find that the fatality between 20—40 is about 11·7 per cent. for the Leipzig males, and 24·2 per cent. for the London Hospital patients; multiplying the last figure by seven-elevenths, we have 15·4 per cent. as the value corrected on the Stockholm basis, which is still very much above the Leipzig rate. We seem, therefore, to require the aid of another factor, viz., that of social class. The obligatory members of the Leipzig Krankenkasse consist for the most part of skilled workmen in regular employment who have undergone some rough selection from the point of view of bodily fitness; the hospitals of a city like London draw their patients mainly, as we have seen, from the poorest ranks of society, from a class containing a high percentage of casual labourers. These classes possibly differ congenitally in the sense that they are sprung from stocks of unequal fitness, and they certainly differ in their environments, using the word environment in a wide sense. It is possible, even probable, that much of the difference in rate of fatality can be attributed to the whole of these inherited and acquired conditions—in a word, that the figures provide us with a rough arithmetical measure of the disease-resisting powers of the very poor and of the moderately prosperous.

We have not specifically referred to the effect of nationality, isolated from climatic conditions, on the prognosis of pneumonia for two grounds; in the first place the data from Johns Hopkins Hospital in Baltimore, so far as they go, do not seem to warrant our attaching much importance to nationality *per se;* and in the second place, the data from Austria, Switzerland and Sweden when combined with the English results do not yield any fair basis of argument. We have no material for instituting a comparison between very widely separate peoples, such as the black and white races.

Whether our speculations respecting the source of the discrepancy we have noted be or be not correct, it is at any rate true that our results afford a striking illustration of the incorrect ideas respecting case mortality which might be derived from an exclusive study of hospital records. We must not prolong this paper by a further discussion of speculative ideas.

While our inquiry has been in some directions practically barren of results and we have by no means realised the expectations with which we embarked upon it, yet it may be that a part of the material presented is not without interest to the statistician and we hope at least to have spared subsequent inquirers some fruitless toil.

Summary of main conclusions.

1. The fatality of simple fractures of the leg diminished greatly and almost steadily down to fifty years ago, since then the change has not been regular.
2. There is a slight but probably significant difference between the male and female rates.
3. The fatality of compound fractures has diminished steadily down to the present time, but the data are imperfect.
4. The hospital fatality of lobar pneumonia has changed little during the last fifty years, and there is no sexual difference in the rates which can be regarded as statistically significant.
5. The experience of different London hospitals is concordant but great discrepancies exist on extending the comparison to other countries.
6. The change in fatality with age can be roughly represented by a straight line.
7. The apparently lower rate of fatality among patients not treated in hospitals is, perhaps, to a large extent due to differences in social class, but to settle the point we should require a long series of English cases from the classes not treated in hospitals.[17]

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[17] I am much indebted to my friend Mr. G. Udny Yule, who kindly went through the whole of the MS. and suggested several important modifications.—M.G.

APPENDIX.

Table I.—*Simple fractures of the leg*, 1751-1903.*

Years.	Totals.	Deaths.	Percentage fatality.	Mean age.*	Standard deviation of ages.	Coefficient of variation.
1751-62 (only '61-62 give ages)	399	29	7·27	37·9	16·4	43·3
'63-72	565	51	9·03	37·3	14·9	39·9
'73-82	442	14	3·17	35·9	16·3	45·4
'83-92	457	11	2·41	36·6	16·4	44·8
'93-1801 ('96, '98, 1900, and half '01 missing)	225	11	4·89	38·5	15·6	40·5
1814-23	480	22	4·58	37·4	17·4	46·5
'24-33	674	18	2·67	36·3	17·3	47·7
'34-43	992	25	2·52	36·9	16·0	43·4
'44-53 (– '47)	1,271	24	1·89	34·7	16·5	47·6
'54-63 (– '54 and '63)	1,266	11	0·87	33·4	16·4	49·1
'64-73 (–'64, '65, '72, '73)	1,459	15	1·03	34·3	17.0	49·6
'74-83 (middle 4 years)	1,317	13	0·99	33·9	17·1	50·4
'84-93 (middle 4 years)	1,203	4	0·33	35·1	16·3	46·4
'94-1903 (middle 4 years)	1,154	7	0·61	35·6	17·1	48·0

* The age grouping used ran: 1—5, 6—15, 16—25, &c. In calculating means it was assumed that the age entries refer to age at last birthday so that a person entered as *m* years was on the average $m + \cdot 5$ years old. Later tables are usually given with groupings comparable with those of most *medical* writers. It was found on a sample of 3,426 cases that using the unsatisfactory grouping 1—10, 11—20, &c., did not appreciably alter the values. In any case the figures are only of interest for comparative purposes as age records in this type of material are notoriously inaccurate.

Table II.—*Simple fractures of the leg.*

Males only.

Years.	Number of cases.	Number of deaths.	Percentage fatality.		Mean age.	Standard deviation of ages.	Coefficient of variation.
1844–53	1,066	18	1·7	1·13	33·4	15·8	47·3
'54–63	1,044	9	0·86				
'64–73	1,166	10	0·86				
'74–83	1,039	9	0·87	0·64	33·1	16·3	49·2
'84–93	916	4	0·44				
'94–1903	865	5	0·59				

Table III.

Females only.

1844–53	205	6	2·9	1·81	36·8	20·1	54·6
'54–63	222	2	0·9				
'64–73	293	5	1·7				
'74–83	278	4	1·4	0·70	39·5	17·6	44·6
'84–93	287	—	—				
'94–1903	289	2	0·69				

TABLE IV.—*Correlation between age and severity in simple fracture cases.*

Years.	Number of cases.	Correlation.	Years.	Number of cases.	Correlation.
A. ALL CASES.			B. MALES ONLY.		
1761–92	1,534	·21 ± ·05			
'93–1843 ...	2,368	·30 ± ·04			
1844–73	3,998	·48 ± ·03	1844–73........	3,277	·41 ± ·03
'74–1903	3,674	·31 ± ·03	'74–1903....	2,820	·37 ± ·03

TABLE IVA.—*Case mortality per* 1,000. *Corrected for ages, the admissions* 1884-1903 *being taken as the standard population.*

Years.	Case mortality per 1,000.	Case mortality divided by the standard rate.	Years.	Case mortality per 1,000.	Case mortality divided by the standard rate.
1751–82........	62·191	13·3	1854–83........	10·294	2·2
'83–1823....	38·080	8·2	'84–1903....	4·667	1·0
1824–53........	19·918	4·3			

TABLE V.—*Age-distribution of males,* 1831-1903, *admitted with fracture of tibia or fibula (exclusive of Pott's fracture*).*

Age Group.	Tibia.	Fibula.	Age Group.	Tibia.	Fibula.
1— 5	143	19	56—60	33	72
6—10........	243	61	61—65	27	34
11—15........	170	58	66—70	6	21
16—20........	100	109	71—75	4	7
21—25........	101	253	76—80	3	1
26—30........	106	337	81—85	1	0
31—35........	105	285			
36—40........	127	279	Totals	1,390	2,036
41—45........	98	209			
46—50........	64	184	Means	25·7 years	35·1 years
51—55........	59	107			

* All such fractures are due to combined eversion and abduction of the foot.

Table VI.—*Age graduation of fractures of fibula, males,* 1831-1903.

Age.	Observed.	Calculated.*	Age.	Observed.	Calculated.*
1— 5........	19	14	46—50	184	175
6—10.... ..	61	38	51—55	107	114
11—15........	58	84	56—60	72	66
16—20........	109	151	61—65	34	32
21—25	253	227	66—70	21	14
26—30........	337	283	71—75	7	6
31—35.......	285	305	76—80	1	2
36—40........	279	286	81—85	0	1
41—45........	209	236			

* The constants deduced from the observations by the methods of moments were :—

$$\beta_1 = {\cdot}03997,\ \beta_2 = 2{\cdot}90929,\ \kappa_2 = -\ {\cdot}10055,\ r = 37{\cdot}21879,$$

the equation to the frequency-curve was :—

$$y = 306{\cdot}91\left(1 + \frac{x}{11{\cdot}6427}\right)^{11{\cdot}9845} \times \left(1 - \frac{x}{22{\cdot}5718}\right)^{23{\cdot}2343} \text{ the skewness } = {\cdot}1132.$$

Mode at 33·58, Mean at 35·1 years.

Table VII.—*Age-distributions of* 865 *male and* 289 *female patients admitted for simple fractures of the leg,* 1897-1900, *compared with the age constitution of Stepney* (*Census of* 1901).

(Numbers expressed as percentages.)

Age Group.	Males.		Females.	
	Fractures.	Census.	Fractures.	Census.
0— 4	3·35	13·23	5·54	13·45
5— 9	10·40	11·23	6·23	11·65
10—14	4·74	10·15	2·77	10·28
15—19	3·93	9·84	2·08	10·32
20—24	4·51	10·45	3·46	10·63
25—29	8·55	9·21	7·96	8·80
30—34	12·72	7·91	9·00	7·23
35—39	13·18	6·87	9·00	6·29
40—44	11·45	5·60	11·42	5·13
45—49	11·21	4·37	13·49	4·20
50—54	7·17	3·78	9·00	3·60
55—59	3·47	2·58	5·54	2·57
60—64	3·24	2·12	9·69	2·30
65—69	1·15	1·26	2·08	1·54
70—74	0·58	0·79	1·73	1·09
75—79	0·23	0·43	1·04	0·63
80—84	0·12	0·16	—	0·29

TABLE VIII.—*Simple fractures of the thigh*, 1751-1903.

Years.	Total.	Deaths.	Percentage fatality.	Mean age.	Standard deviation of ages.	Coefficient of variation.
1751-62 (only '61, '62 give ages)	161	15	9·3	32·4	18·2	56·2
'63-72	156	6	3·9	32·3	20·3	62·8
'73-82	200	12	6·0	30·0	20·2	67·3
'83-92	153	3	2·0	30·4	19·4	63·8
'93-1802(5½ years missing)	95	4	4·2	32·5	21·9	67·4
1814-23	269	16	6·0	33·3	22·1	66·4
'24-33	343	36	10·5	36·3	24·4	67·2
'34-43	470	37	7·9	36·0	25·1	69·7
'44-53 ('47 missing)	554	25	4·5	31·3	26·1	83·4
'54-63	743	30	4·0	30·2	25·4	84·1
'64-73	930	26	2·8	29·1	25·0	85·9
'74-83	1,044	61	5·8	29·5	25·8	87·5
'84-93	922	30	3·3	29·6	26·0	87·8
'94-1903	833	14	1·7	28·8	25·7	89·2
Total	6,873	315	4·6	—	—	—

TABLE IX.—*Simple fractures of the thigh.*

MALES ONLY.

Years.	Number of cases.	Number of deaths.	Percentage fatality.		Mean age.	Standard deviation.	Coefficient of variation.
1844-53	403	9	2·2	2·6	26·6	22·4	84·2
'54-63	550	22	4·0				
'64-73	673	12	1·8				
'74-83	726	32	4·4	3·0	25·0	23·0	92·0
'84-93	645	18	2·8				
'94-1903	592	8	1·4				

TABLE X.

FEMALES ONLY.

1844-53	151	16	10·6	6·3	38·3	31·4	82·0
'54-63	193	8	4·1				
'64-73	257	14	5·4				
'74-83	318	29	9·1	5·6	38·4	30·0	78·1
'84-93	277	12	4·3				
'94-1903	241	6	2·5				

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* *Biometrika*, Vol. VI. p. 377.

Table XI.—*Correlation between age and severity in simple fractures of the thigh.*

Years.	Number of cases.	Correlation.	Years.	Number of cases.	Correlation.
A. All Cases.			B. Males only.		
1761–92	537	·24 ± ·08			
'93-1843	1,175	·54 ± ·04			
1844–73	2,224	·52 ± ·03	1844–73........	1,623	·48 ± ·04
'74–1903	2,800	·38 ± ·03	'74–1903....	1,960	·37 ± ·04

Table XII.—*Fatality of compound fractures of the leg. (All cases.)*

Period.	Number of cases.	Number of deaths.	Percentage fatality.
1752–1801	241	100	41·5
1801–50	250	85	34·0
'51–65	278	89	32·0
'66–76	289	65	22·5
'77–90	299	44	14·7
'91–1903	257	9	3·5

Table XIII.—*Pneumonia statistics. London Hospital,* 1784-1903.

Years.	Number of cases.	Deaths.	Percentage fatality.	Mean age.	Standard deviation of ages.	Coefficient of variation.
1784–1800	235	59	25·1	33·4	13·6	40·7
1814–30	186	61	32·8	32·9	12·1	36·8
'31–53	155	40	25·8	32·9	12·1	36·8
'54–63	201	46	22·9	30·1	14·9	49·5
'64–73	254	62	24·4	26·9	14·2	52·8
'74–83	456	126	27·6	27·9	14·7	52·7
'84–93	1,749	348	19·9	24·9	15·1	60·6
'94–1903	2,456	415	16·9	22·6	14·8	65·6
Totals	5,692	1,157	20·3	—	—	—

TABLE XIV.—*Percentage case mortality of lobar pneumonia, London Hospital*, 1831-1903.

No figures are given which are based on less than 20 observations. The probable errors have been calculated by $E = \frac{67\cdot449}{n}\sqrt{pq(n+1)}$. This formula is not satisfactory for small values of p. To call attention to this, the probable errors in doubtful cases are enclosed in brackets.

Age group.	1831-54.		1854-63.		1864-73.	
	Males.	Females.	Males.	Females.	Males.	Females.
0— 5	....	....	....	....	....	....
6—10	....	....	....	....	....	....
11—15	....	....	....	....	....	....
16—20	....	....	10·0±4·6	....	....	....
21—25	....	....	9·4±3·5	....	21·6±4·6	....
26—30	36·4±7·1	....	....	....	14·8±4·7	....
31—35	....	....	....	....	26·1±6·3	....
36—40	....	....	....	....	....	....
41—45	....	....	....	....	....	....
46—50	....	....	....	....	....	....
51—55	....	....	....	....	....	....
56—60	....	....	....	....	....	....
61—65	....	....	....	....	....	....
1—10	....	....	....	....	....	....
11—20	....	....	15·4±4·9	....	12·9±4·1	8·7±4·0
21—30	27·5±4·8	35·0±7·4	10·9±3·1	....	18·8±3·3	9·1±4·2
31—40	24·1±5·5	....	31·4±5·4	....	31·4±5·4	....
41—50	....	....	34·8±6·8	....	....	....
51—60	....	....	....	....	....	....
61—70	....	....	....	....	....	....

Age group.	1874-83.		1884-93.		1894-1903.	
	Males.	Females.	Males.	Females.	Males.	Females.
0— 5	36·7±6·0	....	15·1±2·2	18·9±3·1	7·8±[1·3]	4·5±[1·3]
6—10	....	....	1·8±[0·9]	7·9±[2·3]	1·7±[0·6]	2·7±[1·0]
11—15	5·0±[3·4]	....	4·8±[1·3]	1·9±[1·3]	2·2±[0·7]	3·8±[1·5]
16—20	17·4±3·8	13·6±5·0	10·0±1·5	7·0±[2·3]	7·3±[1·0]	13·8±2·6
21—25	16·7±4·2	....	15·5±2·1	15·0±3·1	10·3±1·4	14·0±3·6
26—30	30·2±4·8	....	28·5±2·5	12·2±3·2	24·1±2·2	16·7±3·7
31—35	20·0±5·0	....	34·6±3·2	29·5±4·7	29·5±2·6	17·9±5·0
36—40	32·4±5·3	....	36·0±3·1	28·6±5·2	40·6±2·8	43·6±5·4
41—45	41·2±5·8	....	42·1±3·8	....	31·8±3·4	40·0±7·6
46—50	....	....	40·3±4·2	....	45·6±4·1	....
51—55	....	....	16·7±4·2	....	58·7±4·9	....
56—60	....	....	....	....	54·8±6·1	....
61—65	....	....	....	....	....	....
1—10	25·5±4·3	17·4±5·4	8·7±1·3	13·9±2·0	4·4±[0·7]	3·6±[·84]
11—20	13·6±2·9	9·7±3·6	7·8±[1·0]	4·5±[1·3]	5·2±[0·7]	8·8±1·5
21—30	24·1±3·3	39·4±5·8	22·2±1·6	13·8±2·2	16·6±1·3	15·4±2·6
31—40	26·9±3·7	30·0±7·1	35·3±2·2	29·1±3·5	35·1±2·0	32·8±3·9
41—50	43·4±4·6	....	41·3±2·8	23·3±5·3	37·8±2·6	42·9±5·7
51—60	61·9±7·3	....	30·1±4·2	....	57·1±3·8	....
61—70	....	....	54·5±7·3	....	78·3±5·9	....

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* *Biometrika*, Vol. VI. p. 377.

TABLE XV.—*Pneumonia*, 1854-1903.

MALES.

Ages.	Totals.	Deaths.	Percentage case mortality.
0— 5	353	50	14·16 ± 1·25
6—10	380	8	2·10 ± [0·497]
11—15	394	15	3·81 ± [0·65]
16—20	551	53	9·62 ± 0·85
21—25	450	60	13·33 ± 1·08
26—30	405	103	25·43 ± 1·46
31—35	314	94	29·94 ± 1·74
36—40	320	121	37·81 ± 1·83
41—45	223	85	38·12 ± 2·19
46—50	165	73	44·24 ± 2·62
51—55	108	43	39·81 ± 3·19
56—60	68	39	57·35 ± 4·07
61—65	46	26	56·52 ± 4·98
66—70	14	9	64·29 ± 8·94
71—	10	6	60·00 ± 10·96
Totals	3,801	785	20·65

FEMALES.

0— 5	207	26	12·56 ± 1·55
6—10	197	12	6·09 ± [1·15]
11—15	155	5	3·23 ± [0·96]
16—20	182	23	12·64 ± 1·67
21—25	141	21	14·89 ± 2·03
26—30	128	24	18·75 ± 2·34
31—35	90	23	25·56 ± 3·12
36—40	87	31	35·63 ± 3·48
41—45	44	16	36·36 ± 4·95
46—50	36	12	33·33 ± 5·37
51—55	28	12	42·86 ± 6·42
56—60	11	5	45·45 ± 10·58
61—	9	2	22·22 ± 9·85
Totals	1,315	212	16·12

TABLE XVI.—*Pneumonia statistics*, 1854-1903. *Sexual differences in fatality.*

Age group.	Sex with higher fatality rate.	Difference and probable error.	Ratio of difference to its probable error.
16—20	Female	3·02 ± 1·87	1·61
21—25	,,	1·56 ± 2·30	0·68
26—30	Male	6·68 ± 2·76	2·42
31—35	,,	4·38 ± 3·57	1·23
36—40	,,	2·18 ± 3·93	0·55
41—45	,,	1·76 ± 5·41	0·33
46—50	,,	10·91 ± 5·98	1·82
51—55	Female	3·05 ± 7·17	0·43
56—60	Male	11·90 ± 11·33	1·05

TABLE XVII.—*Pneumonia—case mortality. Summary of chief published series of hospital statistics.*

[As the crude figures are generally not published, it has not been possible to use the same age grouping throughout.]

LONDON HOSPITAL, 1854-1903 (Greenwood and Candy).

Age.	Total number of cases.		Percentage fatality.	
	Males.	Females.	Males.	Females.
0—10	733	404	7·9	9·4
11—20	945	337	7·2	8·3
21—30	855	269	19·1	16·7
31—40	634	177	33·9	30·5
41—50	388	80	40·7	35·0
51—60	176	39	46·6	43·6
61—70	60	9	58·3	22·2
71	10		60·0	
	3,801	1,315	—	—

ST. BARTHOLOMEW'S HOSPITAL, 1897-1906 (Brown, Roy. Soc. Med.).

Age.	Total number of cases.		Percentage fatality.	
	Males.	Females.	Males.	Females.
Under 15	131	55	2·3	·0
15—20	180	59	4·4	5·1
20—30	210	47	10·5	4·3
30—40	177	44	26·0	20·5
40—50	108	22	30·6	31·8
50—60	48	12	50·0	41·7
Over 60........	11	7	54·5	71·4
	865	246	—	—

GUY'S HOSPITAL,* 1902-06 (Fawcett, Roy. Soc. Med.).

Age.	Total number of cases.	Percentage fatality.
1—10...........	285	6·0
11—20...........	174	7·5
21—30...........	98	22·4
31—40...........	80	28·8
41—50...........	56	41·1
51—60...........	24	41·7
Over 60	10	60·0
	727	—

MIDDLESEX, KING'S COLLEGE, WESTMINSTER (Pasteur and others, Roy. Soc. Med.), 1897-1906.

Age.	Total number of cases.	Percentage fatality.
10—19	306	5·6
20—29	308	14·9
30—39	207	34·3
40—49	191	38·7
50—59	83	57·8
60, &c.	50	66·0
	1,145	—

* Sexes not given separately.

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* *Biometrika*, Vol. VI. p. 377.

TABLE XVII *Contd.—Pneumonia—case mortality.*

ST. MARY'S and CHARING CROSS HOSPITALS, 1897-1906 (Coby and Bosanquet, Roy. Soc. Med.).

Age.	Total number of cases.	Percentage fatality.
10—20..........	180	7·2
21—30..........	204	18·1
31—40..........	139	30·2
41—50..........	79	40·5
51—60..........	26	30·8
61, &c..........	19	52·6
	647	—

ST. THOMAS' HOSPITAL, 1880-90 (Hadden, McKenzie and Ord).

Age.	Total number of cases.	Percentage fatality.
0—10	123	7·3
11—20	182	4·4
21—30	153	22·2
31—40	120	30·8
41—50	85	37·6
51—60	27	55·6
61, &c.	15	66·7
	705	—

MIDDLESEX HOSPITAL (Sturges and Coupland).

Age.	Total number of cases.	Percentage fatality.
0— 9..........	188	2·7
10—19..........	239	4·8
20—29..........	230	17·0
30—39..........	147	31·3
40—49..........	107	47·7
50—59..........	62	40·3
60—69, &c.......	26	38·5
	999	—

JOHNS HOPKINS HOSPITAL, 1889-1905 (Chatard* J. H. H. Reports, xv, 1910, 55).

Age.	Total number of cases.	Percentage fatality.
1—10	47	6·4
10—20	69	17·4
20—30	212	19·3
30—40	150	30·0
40—50	88	43·2
50—60	42	57·1
60—70	30	83·3
70	14	57·1
	652	—

* Including 35 "terminal" pneumonias.

WESTERN INFIRMARY, GLASGOW (J. G. Andrew, *Age incidence, &c., and comparative frequency in disease*, page 235. London, 1909.

Age.	Total number of cases.		Percentage fatality.	
	Males.	Females.	Males.	Females.
1—10	27	11	3·7	9·1
10—20	164	43	4·9	7·0
20—30	254	53	15·0	15·1
30—40	150	45	29·3	31·1
40—50	134	22	39·6	45·5
50—60	58	7	51·7	28·6
60—	27	2	51·9	50·0
Totals	814	183	—	—

TABLE XVIII.—*Pneumonia cases (males) 1854-1903. Graduation of fatality rates.*

Age group.	Actual percentage fatality observed.	Calculated (1).*	Calculated (2).*	Age group.	Actual percentage fatality observed.	Calculated (1).*	Calculated (2).*
6—10....	2·1	—	0·3	41—45	38·1	40·4	39·3
11—15...	3·8	5·3	4·2	46—50	44·2	46·3	45·5
16—20...	9·6	11·2	9·2	51—55	39·8	52·1	51·5
21—25....	13·3	17·0	14·8	56—60	57·4	58·0	57·0
26—30....	25·4	22·9	20·7	61—65	56·5	63·8	61·4
31—35....	29·9	28·7	26·8	66—70 ...	64·3	69·7	62·1
36—40....	37·8	34·6	33·0	70	60·0	75·5	—

* (1) Gives ordinates of the line $y = 5{\cdot}8476\,x - 7{\cdot}1130$. y measured from 30 per cent.; origin of x mid point of group 26—30. (2) Gives ordinates of the skew curve $y = 62{\cdot}711\left(1 + \frac{x}{11{\cdot}801}\right)^{1{\cdot}2474}\left(1 - \frac{x}{{\cdot}7914}\right)^{{\cdot}08359}$

TABLE XIX.—*Age constitution of the male cases of pneumonia admitted 1899-1903 (1,094 cases) compared with the age constitution of the male inhabitants of Stepney.*

[Numbers reduced to percentages.]

Age group.	Pneumonia patients.	Inhabitants of Stepney.	Age group.	Pneumonia patients.	Inhabitants of Stepney.
0— 4.......	10·05	13·23	35—39.......	6·76	6·87
5— 9.......	12·07	11·23	40—44.......	5·30	5·60
10—14.......	11·61	10·15	45—49.......	3·29	4·37
15—19.......	15·90	9·84	50—54.......	2·93	3·78
20—24.......	12·34	10·45	55—59.......	1·83	2·58
25—29.......	8·78	9·21	60—64.......	1·10	2·12
30—34.......	7·13	7·91	64...............	0·91	2·64

TABLE XX.—*Pneumonia fatality statistics.*

Data other than hospital records.

Ages.	Total number of cases.		Number of deaths.		Percentage fatality.	
	Males.	Females.	Males.	Females.	Males.	Females.
*A. Collective Investigation Committee of Brit. Med. Assoc.**						
—10........	107	59	9	4	8·4	6·8
11—20........	144	74	6	8	4·2	10·8
21—30........	143	72	15	12	10·5	16·7
31—40........	110	56	23	12	20·9	21·4
41—50........	90	31	31	7	34·4	22·6
51—60........	47	24	16	9	34·0	37·5
61—70........	35	24	11	9	31·4	37·5
71—	11	12	6	8	54·6	66·7
Totals	687	352	117	69	17·0	19·6

* C. I. R., ii, 29.

TABLE XX *Contd.—Pneumonia fatality statistics.*

Ages.	Total number of cases.		Number of deaths.		Percentage fatality.	
	Males.	Females.	Males.	Females.	Males.	Females.
B. *Versicherungspflichtige Mitglieder of the Leipzig Krankenkasse.**						
—15	25	—	0	—	—	—
15—19	596	—	23	—	3·9	—
20—24	459	—	32	—	7·0	—
25—29	438	—	56	—	12·8	—
30—34	417	—	50	—	12·0	—
35—39	392	—	61	—	15·6	—
40—44	361	—	62	—	17·2	—
45—49	281	—	57	—	20·3	—
50—54	187	—	47	—	25·1	—
55—59	130	—	43	—	33·1	—
60—64	93	—	34	—	36·6	—
65—69	55	—	22	—	40·0	—
70—	38	—	13	—	34·2	—
Totals	3,471	—	500	—	14·4	—

* *Krankheitsverhältnisse f. Leipzig, &c.* Berlin: Heymann, 1910.

TABLE XXI.—I. *London Hospital pneumonia statistics* (*males* 1854-1903). *Regrouped to admit of comparison with the Leipzig data.*

Ages.	Cases.	Deaths.	Percentage fatality.	Ages.	Cases.	Deaths.	Percentage fatality.
— 4	281	45	16·01	45—49	170	62	36·47
5— 9	385	12	3·12	50—54	132	60	45·45
10—14	362	13	3·59	55—59	74	43	58·11
15—19	545	40	7·34	60—64	50	26	52·00
20—24	471	61	12·95	65—69	19	10	52·63
25—29	412	96	23·30	70	12	8	66·67
30—34	325	101	31·08				
35—39	310	109	35·16				
40—44	253	99	39·13		3,801	785	—

II. *Comparison of London Hospital and Leipzig fatality rates for five large groups.*

Age group.	Difference.	Probable error of difference.	Ratio of difference and probable error.	Age group.	Difference.	Probable error of difference.	Ratio of difference and probable error.
20—24	5·98	1·32	4·53	35—39	19·60	2·21	8·87
25—29	10·51	1·77	5·94	40—44	21·96	2·46	8·93
30—34	19·09	2·04	9·36				

TABLE XXII.—*Graduation of the Leipzig fatality rates.*

Column (1) gives the observed percentages.

Column (2), ordinates of the best straight line, viz., $Y = 3{\cdot}74 + 3{\cdot}144X$, where Y is measured from the fatality at ages 15—19, and the unit of X is 5 years.

Column (3) ordinates of the skew-curve. $y = 3{\cdot}3919(x + 13{\cdot}2683)^{1{\cdot}0049}(-x)^{-{\cdot}0521}$. Origin at the end of the range, which extends from 11·2 to 77·6 years, the unit being 5 years.

Ages.	(1.)	(2.)	(3.)	Ages.	(1.)	(2.)	(3.)
—15....	0	0·60	0·74	45—49	20·28	22·61	22·58
15—19....	3·86	3·74	3·73	50—54	25·13	25·75	25·96
20—24....	6·97	6·89	6·76	55—59	33·08	28·90	29·45
25—29....	12·79	10·03	9·83	60—64	36·56	32·04	33·15
30—34....	11·99	13·18	12·93	65—69	40·00	35·18	37·19
35—39 ...	15·56	16·32	16·09	70—	34·21	38·33	41·97 (ages 70—74)
40—44 ...	17·17	19·46	19·30				

TABLE XXIII.—*Statistics of the Seraphim Hospital, Stockholm*, 1840–55 (*Huss.*)

All cases.				*Excluding patients admitted in a moribund state.*			
Ages.	Number of cases.	Number of deaths.	Percentage fatality.	Ages.	Number of cases.	Number of deaths.	Percentage fatality.
5—10....	9	1	11·1	5—10	9	1	11·1
10—20....	233	18	7·7	10—20	229	14	6·1
20—30....	1,064	84	7·9	20—30	1,041	61	5·9
30—40....	847	128	15·1	30—40 ...	816	97	11·9
40—50....	382	91	23·8	40—50 ..	363	72	19·8
50—60...	134	36	26·9	50—60	125	27	21·6
60—70...	36	14	38·9	60—70	29	7	24·1
70—80....	5	3	60·0	70—80	4	2	50·0
Totals ...	2,710	375	13·8		2,616	281	10·7

TABLE XXIV.—*Stockholm cases* (*Huss*). *Sex distribution.*

Ages.	Males.			Females.		
	Cases.	Deaths.	Percentage fatality.	Cases.	Deaths.	Percentage fatality.
5—10........	6	1	16·7	3	—	—
10—20........	195	8	4·1	34	6	17·7
20—30........	901	48	5·3	140	13	9·3
30—40........	714	78	10·9	102	19	18·6
40—50........	284	60	21·1	79	12	15·2
50—60........	69	17	24·6	56	10	17·9
60—70	16	5	31·3	13	2	15·4
70—80........	2	2	100·0	2	—	—
Totals	2,187	219	10·01	429	62	14·5

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* *Biometrika*, Vol. VI. p. 377.

DISCUSSION *on* MR. GREENWOOD *and* MR. CANDY'S PAPER.

MR. YULE said he had much pleasure in proposing a vote of thanks to Mr. Greenwood and Mr. Candy for bringing this Paper before the Society. The authors deserved, indeed, a particularly hearty vote of thanks because, judging from the conclusion of the Paper, they seemed to feel somewhat depressed at the lack of definiteness in their results; they suggested that their enquiries had been in some directions barren, with the result that Mr. Greenwood and his colleague had by no means realised the expectations with which they embarked on the enquiry. But the value of a paper ought not to be judged simply from the definiteness of the results obtained. They had at the London Hospital a record of unequalled length as regards the data afforded. It had no doubt occurred to many that such data required investigation, that a number of useful and interesting results ought to be deduced from such data; but nobody seemed to have undertaken the task of investigation, and it was left to Mr. Greenwood and his colleague to undertake this research. They could not tell until the enquiry had made considerable progress how far the data would show striking changes, or how far they might only give results of most doubtful interpretation. It was a great deal to have at least exhibited clearly what were the difficulties in interpreting such data, and possibly to suggest some directions in which they might be improved for comparative purposes. How great were the difficulties of comparison was illustrated by passage after passage in the Paper. First, there were difficulties arising from the selective character of the data. The patients were selected from a special social class; and the intensity of such selection might have changed during the very long period under review. As stated at the beginning of the Paper, the percentage of labourers recorded in the admission books was 23 per cent. at the beginning of the period, and 17 per cent. at the end. But this difference was fairly considerable—nearly one-third of the latter figure—and it certainly raised some doubt how far the data at the beginning and the end of the period were strictly comparable as regards social class. Again, there was a certain selection of cases, probably to an unknown extent, according to severity; the less severe cases might not have gone to the hospital. There was, again, apparently the possibility of selection according to sex, so that one sex might not appear in its proper proportion, *e.g.*, in pneumonia cases. Again, the methods of diagnosis had changed, and were no doubt much better now than they used to be; and that might introduce a whole series of sources of error in making a comparison over a long period. Then, again, the age and sex composition of patients might have changed; and that introduced a further source of difficulty in comparison between the beginning and the end of the period under review; but that difficulty could to a certain extent be obviated by well-known methods. In the case of fractures of the leg there was another difficulty of quite a different kind to which attention was drawn, namely, that the death might not be due to the fracture, but to shock; that introduced a source of error which had to be allowed for in some way. Again, fractures of the leg might be excluded from the records (of amputation cases) in certain cases. Finally, the mere case mortality might be misleading as a guide to what they actually wanted to know, viz., the success of surgical or medical treatment, because, although death might not have resulted, it did not necessarily follow that the patients were discharged as thoroughly cured in the olden days as in the present. Therefore the problem of comparison bristled with difficulties. Beyond all these difficulties, but by no means the least, they had the question, which the authors had dealt with at considerable length, of "fluctuations of sampling," as they were termed. Even if the general conditions remained precisely the same from year to year, one would nevertheless expect more or less considerable fluctuations in the number of deaths from any cause for precisely the same reasons as one expected variations in the proportion of sixes in throwing dice. The magnitude of such fluctuations in the case of dice-throwing could be foretold on theoretical grounds; and if anybody were sceptical as regards the theory he would find that actual experiments had been carried out for checking theory by making long series of throws. In certain forms of mortality the fluctuations one got from year to year were measured with beautiful accuracy by a formula based on the simple theory as applied to dice-throwing. A very good case was one given by von Bortkewitsch, viz., the numbers of deaths in certain German Army Corps from the kick of a horse; it was a particularly simple case, because the number of men and all the conditions were fixed. The fluctuations in deaths from year to year in that case were measured almost precisely to the second place of decimals by the theoretical formula. But that simple theory did not apply to the ordinary population of both sexes and varied ages, even in the case of pure accidents, and there were further risks in applying it when dealing with many causes of death which were not pure accident. When throwing well-made ordinary dice the chance of getting a six was approximately the same for every die at every throw, and the theory was simple. But if one had a lot of bad dice, or possibly a set which one had collected for one's own private purposes, in which the chance of throwing a six with certain dice was very high and the chance of throwing a six with others was very low indeed, and one used a handful of such dice for throwing, the fluctuations in the proportion of sixes thrown would not be that given for the simple case of perfect dice. The population subject to the risk of mortality was like that handful of heterogeneous dice, for the two sexes and individuals of different ages were of quite different susceptibility to many causes of death, and probably many persons even of the same age and sex possessed organically different susceptibilities. With that form of heterogeneity the fluctuations of sampling would be rather less than would be expected in the simple case. But suppose that while the dice were bad, in the same sort of way, one took them up differently; suppose one began by making a series of throws with dice which were loaded so as to give sixes too frequently, then one went on to another series of throws with dice loaded to give sixes not so often as they should, and then with another series of ordinary good dice, and one pooled all those records together. In that case one would get a much higher fluctuation in the proportion of sixes than if one had used the good dice throughout. Again, that represented what happened in the deaths from various causes from year to year; in one year the weather might be favourable, and in another year it might be unfavourable. Again, there was yet another cause of divergence from simple theory. If one threw one die and one got a six, one was no more likely to get a six on throwing the next time. But that was not so if one had a case of infectious disease; if one had one case in a district in a certain period one was more likely to have another. In certain forms of accident even, *e.g.*, explosions in mines, if there was one death there was likely to be more than one. In such cases the events were not independent. All these sources of divergence rendered the applicability of the simple theory of sampling to test the significance of a certain death-rate based on a limited number of observations exceedingly difficult; the subject required a great deal more discussion than it had hitherto received, and he was very glad Mr. Greenwood and Mr. Candy had initiated such a discussion in this Paper. The question was raised nearly thirty years ago by Westergaard, who used in his book on mortality the simple binomial formula for testing the significance of death rates, but the subject had not received much attention in other quarters. As Mr. Greenwood had very well stated, the difficulties were objective difficulties—to determine precisely what they wanted to know, and how they were to represent the case theoretically in order to obtain some mathematical expression for the fluctuation of sampling under the conditions subsisting in fact and not in the usual simplified theory. Even in one of the authors' cases, where he would have expected a better result, it appeared that they had not got by any means the fluctuations of the simplest case of sampling alone; he referred to Table 8, "Fractures of the thigh," which the authors passed over rather briefly in one paragraph because the fluctuations in the fatality were so great. Inconveniently rather for theoretical purposes, the numbers in the first column of the table, on which the mortality was based, fluctuated a great deal themselves, so that no great weight could be attached to the theoretical result; but so far as the figures went, the standard deviation of the observed fatality rates was about twice as great as would have been expected from the theory of sampling in its simplest form. Why did the fatalities fluctuate so much in that case? Had Mr. Greenwood any reason to suggest? He would also like to ask one question with regard to the method used for excluding cases of simple fracture where death might be due to shock. About what proportion of cases had to be excluded owing to death taking place during the first three days, and had that proportion greatly changed during the long period under review?

Dr. Dudfield, in seconding the vote of thanks, said he agreed with Mr. Yule that the thanks of the Society were the more due to the authors in that they had submitted their Paper in spite of the disappointing results which they had obtained from their inquiry. He thought the time had come for systematic examination of hospital statistics. The results of the examination of the records of the Glasgow Infirmary had been recently published, and had been noted in the January issue of the *Journal*. Without wishing to question the authors' right to decide how they should deal with their data, he had one criticism to offer on the method adopted, a criticism based on medical rather than statistical grounds. He thought that the grouping of the periods was unfortunate in that such grouping had been made without reference to the epochal periods of medicine and surgery. Having regard to the great changes in surgery brought about by the introduction of anæsthetics and "Listerism," *i.e.*, antiseptic (or preferably "aseptic") methods of treatment, he would have liked to have seen the data relating to fractures grouped into periods which contrasted the results obtained before and since those changes. In the old days when such injuries were dealt with without anæsthetics, shock, apart from that due to the injury itself, played no small part in the after history of the case. The treatment of compound fractures prior to aseptic surgery yielded very different results to those now obtained. Even with the arrangement adopted by the authors, the figures given in Table I (simple fractures) and Table XII (compound fractures) indicated that such improvements in treatment had not been without effect on the fatalities. Turning to pneumonia, the change in the method of treatment had been nearly as important, bleeding, which was the rule, being now practically abandoned. Instead of doing everything which might lower the patient's resistance to the disease, every effort was made to strengthen his powers of resistance. That change in treatment he believed might be said to have been introduced somewhere between 1855 and 1865. But no matter how the data were handled there still remained a question as to the value of the data themselves. The authors said that the diagnoses on which their figures were based had been taken from the "admission books." It was desirable to know who kept those books, and what diagnosis had been entered therein. Was it the diagnosis made on admission to the hospital? Or was it the diagnosis made after observation of the case? The two were very frequently quite different. He might observe parenthetically that the two diseases chosen for examination were not diseases in which the fatality was deemed to be high, certainly not in the case of healthy young adults. The distribution as regards sex and age was, he thought, vitiated by two facts. In the first place, it was common knowledge that men were more ready to avail themselves of institutional treatment than women. To some extent that was due, in all probability, to the desire to preserve the life of the breadwinner of the family, whose death meant the break-up of the home, which could be carried on somehow even after the death of the mother. Moreover, the mother if she remained at home could do something to keep the home in order during her illness, unless she were very seriously incapacitated. Ages and occupations were well-known to be very unreliably stated, especially by hospital patients. It seemed, indeed, that all statistical data in connection with hospitals, except the actual diagnoses, or what were better still, post mortem examination results, required to be dealt with very circumspectly.

Mr. GREENWOOD in reply, having thanked Mr. Yule and Dr. Dudfield for their kind remarks, said the object of the Paper had been entirely fulfilled by what they had said. The fact was that hospital statistics were in many respects bad, but all the statistical knowledge that the medical student received was based entirely on those statistics; for, in the ordinary text-book which a young man going in for medicine read, the statistics of the fatality of disease were taken from, say Smith's series at St. Bartholomew's, or Jones's series at the London Hospital, and so on. It was therefore necessary to examine them. Another point was that, until one examined them at some length and produced cut and dried evidence as to where they were bad and might be improved, they were not going to be improved. The class of statistics discussed in the Paper was, perhaps, the best of them, strange as it might sound. If one took the statistics dealing with the inheritance of disease they seemed to a statistician perfectly humorous in their badness, and he did not believe they would ever be improved till some body like that Society—perhaps by means of a committee on the subject—made some pronouncement. Most hospital administrators would naturally retort to critics of their records, "You are abusing these things, but why don't you look into them?" Nobody ever looked into them or proposed any reform in the system. With regard to the points raised by Mr. Yule, they were purely on the statistical side, and they were therefore to him personally of extreme interest. Mr. Yule and he had had some talk over the Paper, and he intended to have much further talk with him on the subject. The problem of random dispersions in the case of a varying probability was an exceedingly troublesome thing to deal with statistically, but was very important, not so much in the cases dealt with as in the case of acutely infectious diseases, such as plague. This was a subject which came in his way, for he had had to investigate the statistics of plague. He would have, for instance, returns from an Indian province, and he would find that returns from certain villages showed a much greater mortality than the average of the whole district during a given epidemic. The problem was whether one could attribute this to local conditions, or whether it was an error in sampling. Then there came in all the difficulties which Mr. Yule had raised. His impression was that the only practicable way of dealing with the point was to treat the epidemic as a series of tests with the different sets of dice. The chance of death or of infection must be a function of at least two variables; of the exhaustion of susceptible people, and of the change in the virulence of the organism. The only way he could see of getting at it for the purpose of a practical test was to have the mortality from the epidemic divided up into very short intervals of time, and to compare the rate, so to speak, for each week, which could be roughly paralleled to a different series of sets of dice. The population at risk each week and the chance of infection during that week being known, it was possible to treat the figures by relatively simple methods which he need not go into. This was not very satisfactory from the mathematical standpoint, but it was an important statistical problem requiring immediate consideration. With regard to the fluctuations of the statistics of thigh fractures which Mr. Yule had referred to, the reason he did not feel inclined to attach much importance to them was the enormous variation in the age distribution from decennium to decennium; he strongly suspected that a fracture of the thigh might not be a single entity. Some of these cases were fracture of the neck of the bone in old people, others fracture of the shaft. Strictly speaking, they might be quite different classes of injury, which could not be compared. He could not say exactly the number which were excluded by the three-day rule, but it was not, he thought, very large. He felt quite clear, however, that something of that sort must be done because of the question of shock. With regard to the points raised by Dr. Dudfield most of them were medical; and he felt a certain diffidence in replying to them, because although he was a medical man in the technical sense, his name being included in the Medical Register, he did not think any "patients" of his could now be seen alive. As to the grouping in arbitrary periods of years he was not quite clear that that had made much difference, because those great improvements in medical treatment mentioned by Dr. Dudfield (he could only speak with regard to the London Hospital) had not been introduced at any hard and fast period. To take Listerism as an example, at the time he (Mr. Greenwood) was a student everyone practised the correct ritual; but when his father was a student, thirty to forty years ago, some of the surgeons practised the proper ritual of those days, with the carbolic spray always going; others, he believed, practised the old methods, operating in frock coats which had seen many a bloody day for years, while some tried a half-and-half procedure, so that it took many years for the process to become generalised; and he did not think there were any very sharp chronological lines of division. With regard to the treatment of pneumonia he would have thought that the custom of bleeding might have been given up rather earlier than Dr. Dudfield suggested. He thought it went out, as far as routine practice was concerned, in the fifties. As to diagnosis that was a point he tried to look into, and he was disposed to come to the paradoxical conclusion that the *recorded* diagnosis in the earlier days might be more accurate than at present. In the eighteenth and early nineteenth centuries the house staff, as it was now called, was non-existent; there were no house physicians or house surgeons, but there was a resident medical officer,[1] a man who held that

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[1] The apothecary of the early days; the title of resident medical officer was first used in 1854.

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* *Biometrika*, Vol. VI. p. 377.

appointment often for years, and was probably a fairly experienced person. If one looked at the entries in the old registers the diagnoses often contained many technical words. They were not, he thought, the diagnoses of the porter, who entered what occurred to him at the time. He suspected that they were either made by the surgeon, who perhaps attended even more frequently and regularly than now, or by the resident medical officer, who would be a much more experienced person in the long run than the house staff of the present day. When one came to modern times Dr. Dudfield's point was extremely sound; one could not be sure how far diagnoses were made by the receiving room officers, who were generally young men quite freshly qualified, and how far they had the *imprimatur* of the physician or surgeon, but when one took the particular diseases analysed in the Paper it seemed to him that fracture of the leg was a condition which any fairly experienced person was likely to diagnose correctly in most cases. In the case of pneumonia, not having to diagnose cases himself, he could say, with arm-chair confidence, that it seemed a fairly easy thing to diagnose in typical cases; and that was one of the reasons why Mr. Candy and he chose those two—on the ground that the diagnosis would be moderately easy. He quite agreed that the ages were probably very inaccurate, and as to the occupations the same remark applied. That was one reason why he suggested that 17 per cent. as against 24 per cent. might not mean a very great improvement. The occupations seemed to be rather less crudely analysed in the hospital admission books to-day than formerly. One found people described as "bricklayer's labourers" or under some other subdivisions. So far as he could judge, a person now simply described as "labourer" was not doing anything in particular. In the eighteenth century records of all kinds of people were perhaps included as labourers who would now have a special trade description. Some were agricultural labourers, because the hospital probably drew on a larger geographical area in the eighteenth century than it did now. As to the question of the cause of death after fracture a very small percentage of cases should perhaps be regarded as having died as the direct result of the fracture. Such directly conditioned deaths might result from fatty emboli, but were exceedingly rare; and he thoroughly agreed with Dr. Dudfield that hardly anyone died as the result of an ordinary fracture. The reason why so many people died in the old days was, as far as he could judge, and looking at the matter from a modern standpoint, improper treatment. The curious point was that if one looked at the old records, which gave the length of stay in the hospital, simple fracture cases were kept in often for months, sometimes quite colossal periods. What probably happened to them was that they became subject to bed-sores and got into an unpleasant condition generally, becoming liable to catch some of the infectious diseases which are said frequently to have ravaged a hospital in the old days. These deaths were due to the treatment of the fracture, not to the fracture itself. That was one of the reasons why Mr. Candy and he took those cases. That sort of thing did not happen nowadays, and it was interesting to measure the resultant saving in life. It was not perhaps entirely owing to conscious treatment, but partly to the demands for beds in a hospital that they did not keep patients in bed longer than was absolutely necessary. It was possible that the motive for emptying the beds had had a thoroughly sound effect on the chance of recovery in fracture cases. In the old days there did not seem to have been nearly so much pressure on the space; so the people came in with a fracture and stopped in bed for months, which was not, in most cases, good for them. He was very sorry that the results of the analysis were not more interesting, but he hoped he had made good the case as to the grounds for bringing forward a Paper of this kind at the Statistical Society.

HARRISON AND SONS, PRINTERS IN ORDINARY TO HIS MAJESTY, ST. MARTIN'S LANE.

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* Biometrika, Vol. vi. p. 377.

ROYAL STATISTICAL SOCIETY:

AN OUTLINE OF ITS OBJECTS.

THE *Royal Statistical Society* was founded on the 15th of March, 1834, in pursuance of a recommendation of the British Association for the Advancement of Science, its objects being the careful collection, arrangement, discussion and publication of facts bearing on and illustrating the complex relations of modern society in its social, economical, and political aspects, especially facts which can be stated numerically and arranged in tables; and also, the formation of a Statistical Library as rapidly as its funds would permit.

From its inception the Society has steadily progressed. It now possesses a valuable Library of about 50,000 volumes, and reading rooms. Monthly meetings are held from November to June, which are well attended, and cultivate among its Fellows an active spirit of investigation. The Papers read at these meetings, with an abstract of the discussions thereon, are published in the *Journal*, which now consists of seventy-two annual volumes, and forms of itself a valuable library of reference.

The Society has originated and statistically conducted many special inquiries on subjects of economic or social interest, of which the results have been published in the *Journal*, or issued separately.

To enable the Society to extend its sphere of useful activity, and accomplish in a yet greater degree the various ends indicated, an increase in its numbers and revenue is desirable. With the desired increase in the number of Fellows, the Society will be enabled to publish standard works on Economic Science and Statistics, especially such as are out of print or scarce, and also greatly extend its collection of foreign works. Such a well-arranged Library for reference as would result does not at present exist in England, and is obviously a great *desideratum*.

The Society is cosmopolitan, and consists of Fellows and Honorary Fellows, together forming at the present time a body of about *nine hundred* Members.

The annual subscription to the Society is *Two Guineas*, and at present there is no entrance fee. Fellows may, on joining the Society or afterwards, compound for all future annual subscriptions by a payment of *Twenty Guineas*.

The Fellows of the Society receive gratuitously a copy of each part of the *Journal* as published, and have the privilege of purchasing back numbers at a reduced rate. The Library (reference and circulating), and the Reading Rooms are open daily for the convenience of Members.

Nomination Forms, with any further information, will be furnished on application to the *Assistant Secretary*, *Royal Statistical Society*, 9, *Adelphi Terrace*, *Strand*, *W.C.*, *London*.

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the London Hospital.

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* *Biometrika*, Vol. vi. p. 377.

ROYAL STATISTICAL SOCIETY.

LIST OF PUBLICATIONS.

Note.—Sets or separate numbers of the *Journal*, or of the other publications of the Society (if not out of print), can be obtained at the Offices of the Society, or through any bookseller. Fellows may purchase these publications at a reduced rate.

	Price.
Journal (published quarterly)—	
Vols. 1—72. 8vo. 1838-1909	5*s.* each part.*
Journal (published monthly during the session). New Series—	
Vol. 73. 1910	2*s.* 6*d.* each part.
General Analytical Index to Vols. 1—71 of the Journal (1838-1908). In 5 parts. 8vo.—	
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List of Fellows, containing the Rules and Bye-Laws of the Society, and corrected annually to 31st July, is issued gratuitously.

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* Before 1870 the price varied.

[*From Biometrika, Vol. VII. No. 4. November*, 1910.]

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p-19

A BIOMETRIC STUDY OF PHAGOCYTOSIS WITH SPECIAL REFERENCE TO THE "OPSONIC INDEX."

SECOND MEMOIR. ON THE DISTRIBUTION OF THE MEANS OF SAMPLES.

By M. GREENWOOD, Junr., and J. D. C. WHITE.

(*From the Statistical Laboratories of the Lister Institute of Preventive Medicine and the London Hospital.*)

In a recent memoir* we communicated the results of an analysis undertaken with the object of throwing light on difficulties associated with the ordinary method of estimating opsonic indices. The main results of that investigation can be summarised in the following way:

(1) Phagocytic counts have pronouncedly skew frequency distributions and are good examples of Pearson's skew curves.

(2) The means of small samples have likewise a skew distribution.

(3) The use of a thicker bacillary emulsion while diminishing the skewness, does not, under possible experimental conditions, do so sufficiently to allow of testing on the basis of a Gaussian curve.

With respect to (2), we pointed out that results based, as were ours, upon samples extending in no case to more than 2000 cells, were provisional and that we intended to re-investigate this part of the subject with the help of more adequate material.

The present memoir comprises a study of the distribution of a large sample of phagocytic cells, the distributions of the means of sub-samples and some preliminary contributions to the problem of mean distributions in general.

The basis of this work is a count of 20,000 cells made by one of us (J. D. C. W.) who had had 18 months' previous experience in the Inoculation Department of the London Hospital.

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* *Biometrika*, Vol. VI. p. 377.

In undertaking this large count, those methods were adopted which are usually advised in the preparation of films. The mixture, to be subsequently placed on the slides, was made up of three equal parts, (1) serum, (2) corpuscles, (3) bacterial emulsion. The first two were derived from the operator and, in the case of the corpuscles, care was taken that they should be equally distributed, the upper portion of the centrifugalised blood being taken off and thoroughly mixed in a small tube. The bacterial emulsion was prepared as usual and carefully mixed so as to secure the greatest possible homogeneity. Since we desired a large count of a homogeneous character, not a comparison of different mixtures, it was decided to use one pipette only, but of larger calibre than usual, in order to secure the greater amount of mixture requisite for the preparation of a large number of slides. This pipette was throttled for ease of manipulation. The proportions of the three materials were carefully mixed and the pipette placed in an incubator for fifteen minutes, the emulsion having been designed to give an average of three to four bacilli per cell. The pipette was slightly rotated from time to time in order to keep the corpuscles from settling. Sufficient slides being in readiness, the pipette was withdrawn from the incubator, the contents again thoroughly mixed, and films were prepared as quickly as possible. These were then stained with Aniline Fuchsin and Methylene Blue; thereafter cover-slips were applied with Canada Balsam to preserve the slides during the process of counting. The general quality both of films and staining was exceedingly good, very few slides being of an inferior character.

The counting of so large a number of cells as 20,000 was, needless to say, sufficiently laborious, especially in regard to the leucocytes containing more than 10 bacilli apiece. Reasonable accuracy was found possible up to 15 and the few cells containing more than this were marked 16. A mechanical stage was used—the only method, in the operator's opinion, which can secure that the same cell shall not be counted twice over. The only cells omitted were those the outline of which was indistinct, or where for any reason, e.g. the clumping of cells or bacilli, it could not be determined how many bacilli lay in an individual cell; the possible error introduced by this latter criterion will be discussed below.

Definite fragments of bacteria were counted as bacteria; so also were bacilli in definite contact with the periphery of cells. In order to avoid mental prejudice, addition of the rows of figures was postponed generally for days and always at least until the end of the day's work. In this way 20,000 cells were counted in the course of about a month, with occasional intervals. The sheets of figures were then taken and the totals of successive twenty-fives, fifties and hundreds were determined; then the frequency of each number per cell on each sheet of 500 cells was tabulated, this latter process incidentally eliminating any arithmetical mistakes. The frequencies on each of the forty sheets were then added together and the total frequency obtained.

The actual distribution of the twenty thousand cells is set forth in Table I. Proceeding to fit a curve, without using Sheppard's corrections, the constants of

Table II were obtained and Graph 1 shows the theoretical curve and observations. It is evident that, although the curve represents the general trend of the observations with fair accuracy, the goodness of fit as evaluated in the ordinary way is poor. This matter appears to require comment and explanation.

TABLE I.

Actual Frequencies of 20,000 Cells, together with Corrected Values determined on the Basis of a Random Count of 2,000 Cells from the same Material. (See p. 509 *infra.*)

Number of Bacilli per Cell	Original Value	Corrected Value*
0	1428	1402·75
1	2633	2636·57
2	3351	3355·55
3	3556	3560·83
4	2807	2810·81
5	2038	2040·77
6	1430	1431·94
7	1031	1032·40
8	658	658·89
9	407	407·55
10	271	271·37
11	163	163·22
12	103	103·14
13	61	61·08
14	37	37·05
15	17	17·02
16	9	9·01
Totals	20000	19999·95

It will have been noticed that for the purposes of this count we did not enumerate cells which contained clumps of bacteria. Our reason for this omission was that, to the best of our knowledge, workers ordinarily disregard cells the contents of which cannot be resolved into definite bacilli or fragments of bacilli, and we were most anxious to use data so far as possible identical with those employed in actual practice. It was, however, subsequently pointed out to us, that this process was open to serious objection for the following reasons. In the first place, some workers of great reputation never employ for diagnostic purposes mixtures containing an appreciable number of clumps, say more than two or three per cent. In the second place, the exclusion of clumps would tend arbitrarily to heighten the proportion of cells containing 0 bacilli, since cells containing clumps must be regarded as possessing a phagocytic power at least equal to those which contain one bacillus apiece; indeed, Harvey and McKendrick have adduced evidence† which supports a belief that it is rather the number of bacteria than the number of acts of ingestion which should be taken as a measure of phagocytic power.

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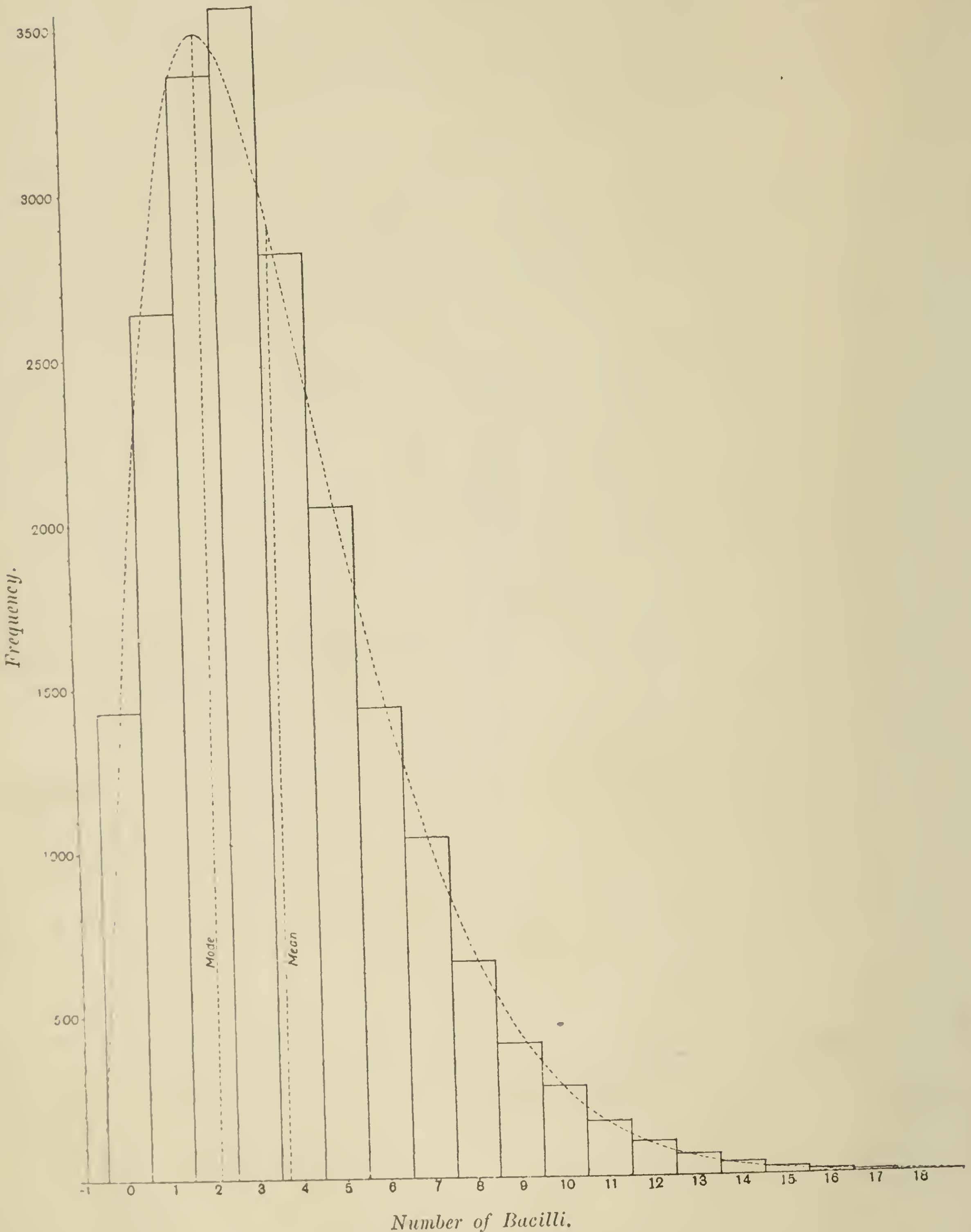
* 1428 cells with 0 bacilli per cell will occur not in 20,000, but in 20,360; hence in 20,000 we shall have not 1428 but 1402·75, the excess is distributed proportionally among the other groups: see p. 509.

† *Biometrika*, Vol. VII. p. 64.

TABLE II.



GRAPH 1. Curve of 20,000 cells.

Constants of Fitted Curve.

$\sigma=2\cdot6031$, $\beta_1=1\cdot0365$, $\beta_2=4\cdot2622$, $\kappa_2=-1\cdot6752$, $r=22\cdot8160$, $b=41\cdot5558$, $m_1=1\cdot3806$, $m_2=19\cdot4354$, $a_1=2\cdot7561$, $a_2=38\cdot7997$, $y_0=3478\cdot81$, Skewness $=\cdot6069$, Mode at $2\cdot1000$, Mean at $3\cdot6797$, Start of curve at $-\cdot6562$, End at $40\cdot8996$, $\chi^2=86\cdot2$, P very small,

$$y=3478\cdot81\left(1+\frac{x}{\quad}\right)^{1\cdot3806}\left(1-\frac{x}{\quad}\right)^{19\cdot4354}.$$

Recognising the force of this objection, it was decided to examine the effect of including clumps in the count. To this end, another 2000 cells were counted on the slides already used and clumped cells were included. Of the 2000 cells counted in this way 36, or 1·8 per cent., contained clumped bacteria. Now if we allow, as seems reasonable, that this latter enumeration was a fair sample of the population, then the frequencies in our 20,000 cells would be changed in the manner shown in the second column of Table I. We have distributed the clumped cells among the other frequencies above zero in accordance with the proportions of those frequencies in the original count, since the small number of clumped cells which were present in the 2000 did not allow us to determine the true proportion of clumps, whether mainly of two, three, or more bacilli*.

The alteration which is effected by this correction is so trifling, that our erroneous method of counting, if it be considered an erroneous method, can hardly have been the cause of the poor fit which resulted, and we have not thought ourselves justified in discarding the data originally collected. Some other source of the poor fit must be looked for.

Other possible explanations are:

(1) The existence of heterogeneity in the material dependent on the fact that cells from different parts of the films are not, or may not be, strictly comparable with each other.

(2) An artificial heterogeneity dependent on the process of counting, other than that already discussed.

It has been noticed before that a large sample of material, even when adequately described by a frequency curve from the diagrammatic point of view, fails to satisfy the approved test. Indeed, many statisticians have been, as Pearson has remarked, far too easily satisfied with the test of mere inspection.

Elderton writes: "I have found in applying the test, that when numbers dealt with are very large, the probability is often small, even though the curve appears to fit the statistics very closely. The explanation is that the statistics with which we deal in practice nearly always contain a certain amount of extraneous matter, and heterogeneity is concealed in a small experience by the roughness of the data. The increase in the number of cases observed removes the roughness, but the heterogeneity remains. The meaning, from the curve-fitting point of view, is that the experience is really made up of more than one frequency curve, but a certain curve, approximating to the one calculated, predominates†."

It will have been noticed that the poverty of fit is mainly due to the cells containing one bacillus being in defect and those containing three in excess; these two groups have added nearly 58 to the value of χ^2. Now the work of counting is excessively monotonous, and after going through some hundreds, it seems impossible to escape an impression that a certain measurement, say 3 bacilli per cell, is modal. Hence a tendency will arise to place any doubtfuls within that particular class. If the count be limited to one or two thousands, the error so introduced may not appreciably affect the result, but it will do so if the data run to many thousands, since the same percentage deviation in a large as in a small experience has naturally a much greater influence on the fit.

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* The 36 cells which contained "clumps" were actually distributed as follows:

Number of Bacilli	Number of Cells	Number of Bacilli	Number of Cells	Number of Bacilli	Number of Cells
1	2	4	4	7	5
2	6	5	8	8	1
3	8	6	0	9	2

† *Frequency Curves and Correlation*, by W. Palin Elderton, p. 142.

The only prejudice to which the operator seems to have been—and thinks he was—subject was the idea which impressed itself on his mind during the count that the number of 3 bacilli per cell occurred with the greatest frequency; this idea may have inclined him to welcome threes with undue alacrity. It is possible that this may have helped in some degree towards producing an excess of threes in the final result. The operator believes that, at least in his case, the auditory sense is more impressed with the sound of numbers containing *e*'s and *i*'s, 3, 5, 9, than with others; he thus tended to believe that these numbers occurred more frequently than was actually the case. Evidently the truth of the matter cannot be exactly known until several independent observers have undertaken the same large count.

We have already mentioned the difficulty regarding clumps; another point is as to whether some gross heterogeneity were produced either by notable differences in various slides or by variations in the method of counting on different occasions, differences of which the operator was unconscious. In making the frequency tables just considered, there emerged necessarily a rough representation of a frequency curve for each five hundred cells. All these curves showed a rough similarity and each one a rough regularity. In some cases, however, the regularity was appreciably less than in others. On going through the data a second time, the groups of 500 which showed the greatest irregularities were excluded. on the supposition that they might represent a somewhat lower grade of accuracy in counting and might have introduced a gross heterogeneity into the total count. The application of this criterion led to the exclusion of 5000 cells. The remaining 15,000 cells were then analysed as before.

Tables III, III A and Graph 2 indicate the results of this process. There is a slight improvement in the fit, but the change is not pronounced. It can hardly be said that the results justify the exclusion.

TABLE III.

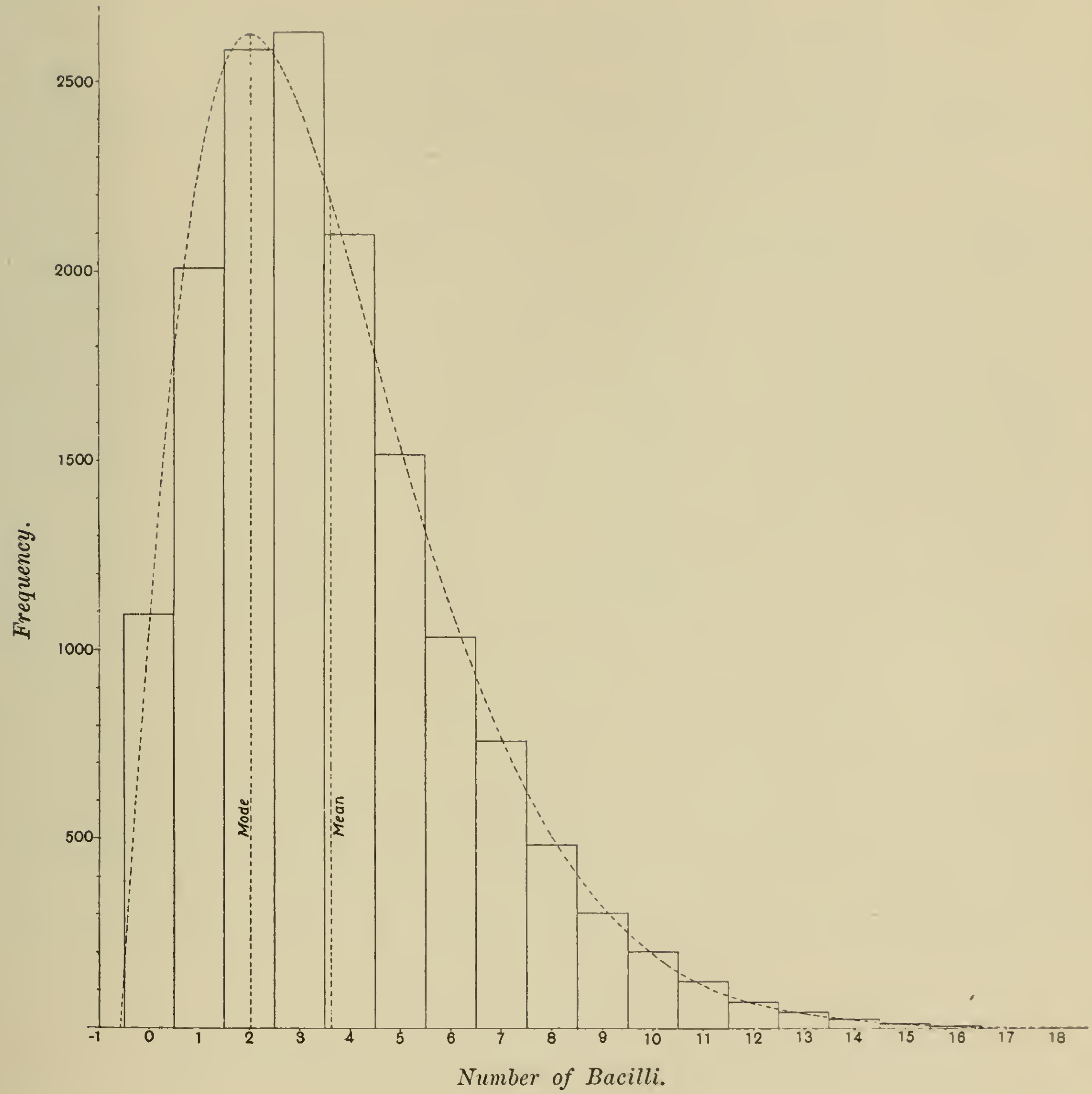
Actual Distribution of 15,000 *Cells.*

Bacilli per Cell	Number of Cells	Bacilli per Cell	Number of Cells
0	1092	*9*	306
1	2008	*10*	203
2	2585	*11*	126
3	2632	*12*	69
4	2096	*13*	44
5	1518	*14*	24
6	1035	*15*	12
7	760	*16*	6
8	484		

Although we should naturally have preferred to obtain closer fits, the result does not seriously affect the value of our material for the purposes of the present investigation. It is clear that the heterogeneity is not caused by a compounding of Gaussian distributions, and that we have to deal with markedly skew variation. We sincerely trust that some other workers may be tempted to repeat the experiment. We now turn to our main inquiry, viz. the problem of sampling.

The 20,000 cells were arranged in *consecutive* 25's, 50's and 100's, and grouped as shown in Table IV. In each case the group is an inclusive one, thus 76—83

TABLE III A



GRAPH 2. Curve of 15,000 cells.

Constants of Fitted Curve.

β_1	β_2	κ_2	r	b	m_1	m_2	a_1	a_2
1·0381	4·2346	−1·5233	20·4319	38·1013	1·2784	17·1535	2·6426	35·4587

y_0	Skewness	Mode	Mean	Start	End	P
2626·615	·61999	2·0360	3·6421	−·5533	37·4947	·00001

$$y = 2626{\cdot}615\left(1+\frac{x}{2{\cdot}6426}\right)^{1{\cdot}2784}\left(1-\frac{x}{35{\cdot}4587}\right)^{17{\cdot}1535}.$$

TABLE IV.

Actually Observed Frequencies of the Samples from 20,000 *Cells.*

Samples of 25		Samples of 50		Samples of 100	
Number of Bacilli per Sample	Number of Samples	Number of Bacilli per Sample	Number of Samples	Number of Bacilli per Sample	Number of Samples
44—51	1	*112—123*	2	*267—282*	2
52—59	14	*124—135*	9	*283—298*	9
60—67	38	*136—147*	21	*299—314*	4
68—75	74	*148—159*	30	*315—330*	13
76—83	128	*160—171*	58	*331—346*	29
84—91	145	*172—183*	83	*347—362*	36
92—99	147	*184—195*	71	*363—378*	34
100—107	113	*196—207*	64	*379—394*	34
108—115	74	*208—219*	31	*395—410*	15
116—123	37	*220—231*	15	*411—426*	11
124—131	19	*232—243*	10	*427—442*	5
132—139	7	*244—255*	3	*443—458*	3
140—147	2	*256—267*	1	*459—474*	1
148—155	0	*268—279*	0	*475—490*	1
156—163	0	*280—291*	1	*491—506*	2
164—171	0	*292—303*	1	*507—522*	1
172—179	1				
Totals	800	—	400	—	200

includes samples with 76 and samples with 83 bacilli per 25 cells. For convenience of calculation, we took an arbitrary origin and unit of grouping in each case. In obtaining the moment coefficients Sheppard's corrections were used in all cases; Table V gives the constants and equations, Graphs 3, 4, 5 illustrate the curves.

TABLE V.

Curves Resulting from an Analysis of the 20,000 *Cells.*

Material	Number of Samples	Mean (in terms of Bacilli per Sample)	Mode (in terms of Bacilli per Sample)	σ (in terms of Bacilli)	β_1	β_2
Samples of 25	800	92·04	89·7488	16·7184	·1033	3·4321
Samples of 50	400	184·07	181·3964	25·7496	·1467	4·0907
Samples of 100	200	367·62	360·2632	39·9824	·3004	4·3500

Material	κ_2	r	m	ν	a	$\beta_2 - 3$	Skewness	P (Fit)
Samples of 25	·1436	25·2068	13·6033	− 10·3220	9·5149	·4321	·1371	·9765
Samples of 50	·0665	10·1436	6·0718	− 2·7070	6·2691	1·0907	·1066	·8673
Samples of 100	·1367	10·1723	6·0862	− 4·0483	7·0316	1·3500	·1840	·1727

Equations:
$$y=23{\cdot}428\left[1+\frac{x^2}{9{\cdot}5149^2}\right]^{-13{\cdot}6034}\times e^{10{\cdot}3220\tan^{-1}\frac{x}{9{\cdot}5149}}.$$

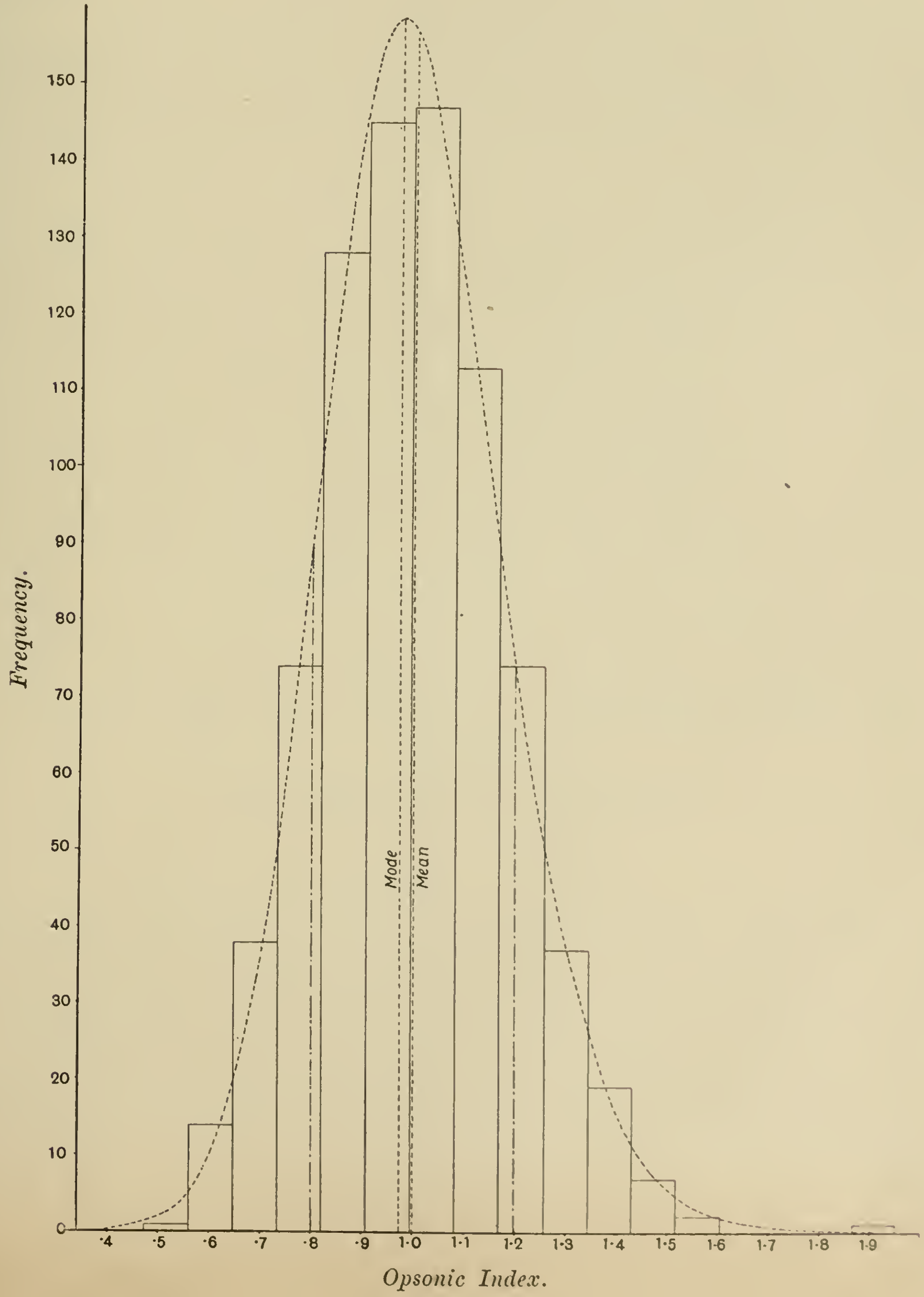
Origin at 2·6712, where 39·5 bacilli are taken as 0 and 8 bacilli per 25 cells form the statistical unit of grouping.

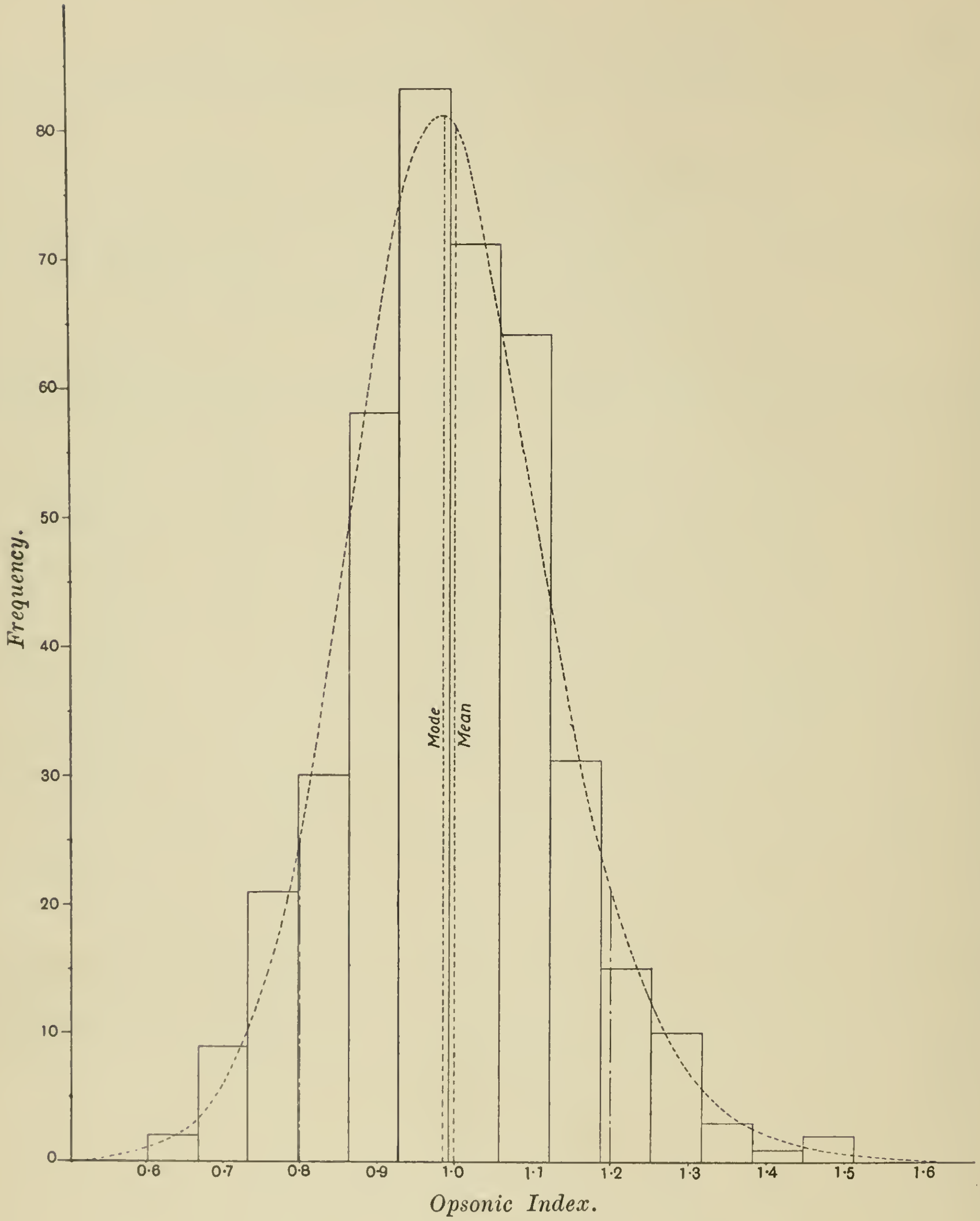
$$y=60{\cdot}047\left[1+\frac{x^2}{6{\cdot}2691^2}\right]^{-6{\cdot}0718}\times e^{2{\cdot}7070\tan^{-1}\frac{x}{6{\cdot}2691}}.$$

Origin at 4·87446, 105·5 bacilli taken as 0 and 12 bacilli per 50 cells as the statistical unit of grouping.

$$y=18{\cdot}117\left[1+\frac{x^2}{7{\cdot}0316^2}\right]^{-7{\cdot}0316}\times e^{4{\cdot}0483\tan^{-1}\frac{x}{7{\cdot}0316}}.$$

Origin at 4·021598, 258·5 bacilli taken as 0 and 16 bacilli per 100 cells as the statistical unit of grouping.





GRAPH 4. Curve of 400 Samples of 50.

The figures below the abscissa represent opsonic indices in terms of the mean of the whole series. That is to say, each group unit was translated back into physical terms and then divided by the mean number of bacilli per sample for the whole count. For instance, in Graph 3, the abscissa ·9 was really 5 in statistical units corresponding to 39·5 (the origin) + 5 × 8 bacilli per 25 cells. The mean is 92·04, therefore the opsonic index in terms of the mean corresponding to this abscissa is $\frac{79{\cdot}5}{92{\cdot}04} = {\cdot}86$ *.

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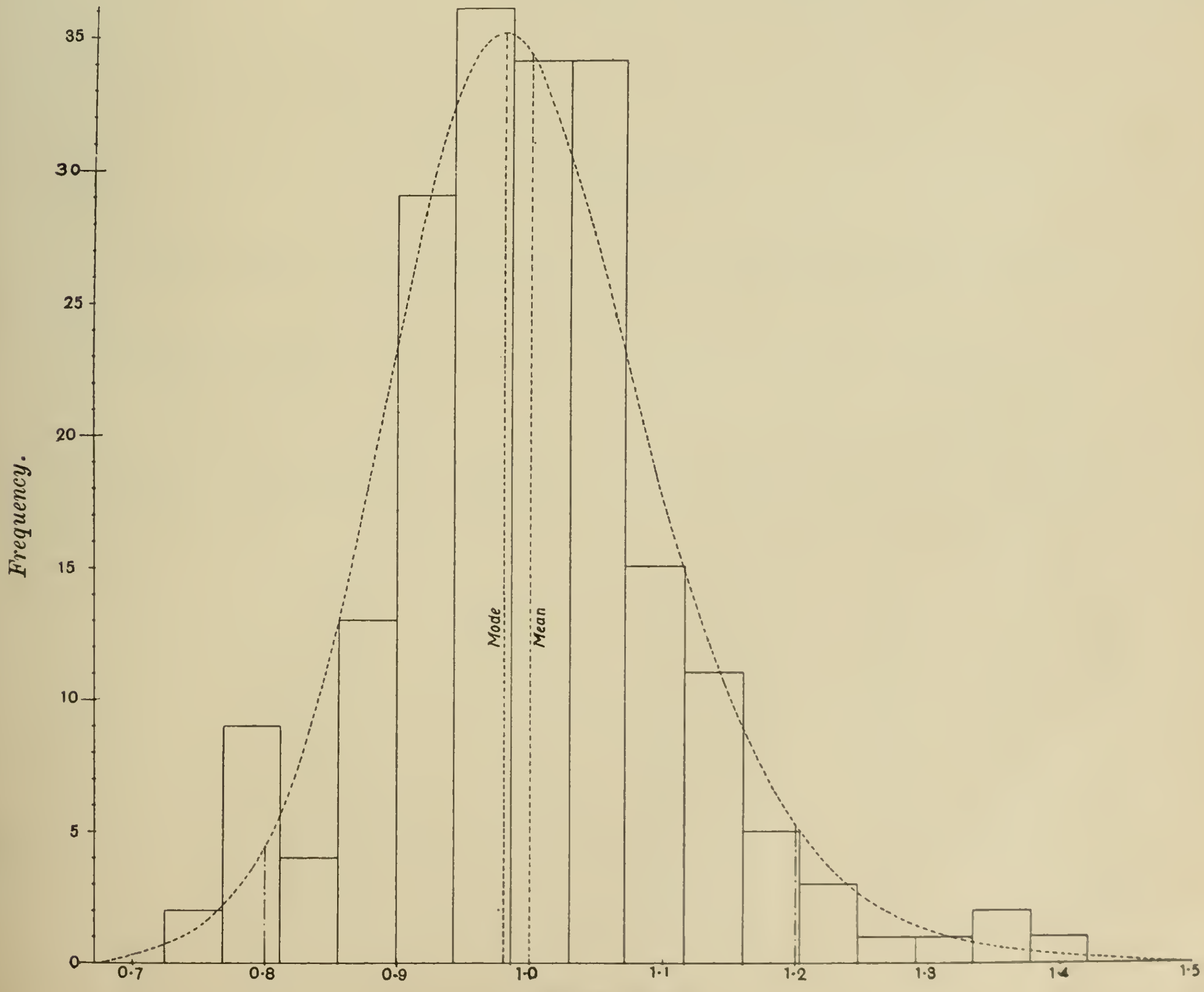
* The indices as plotted are only given to the nearest first decimal.

Objections may fairly be urged against this process. As we have previously remarked, the mean is theoretically not the best constant to use for descriptive purposes in the case of markedly skew frequencies and we suggested the mode as being more satisfactory. Harvey and McKendrick, in an important contribution to the subject*, have alluded to this point and remark with justice that the determination of the true mode requires calculation beyond the range of a laboratory worker. We have considered whether an approximation of sufficient accuracy might not be found. Pearson has shown† that in many cases a good enough value for the mode is given by:

Mode − Median = Twice the Distance from Mean to Median.

Unfortunately, however, the median cannot be determined with sufficient accuracy in the case of discontinuous variates such as phagocytic counts. Thus, we have experimentally no finer measurement than the integral number of bacilli per leucocyte; if we mass in a group all the m individuals containing the same number of bacilli and know that the median is the nth individual in that group, all we can do is to add $\frac{n}{m}$ of the unit of grouping to the next lower integral group measure. This is not nearly close enough in the case of such skew distributions; and we found that the value of the mode thus obtained differed materially from the real mode. It was not therefore possible to use this process with success in the case of our sample curves.



GRAPH 5. Curve of Samples of 100.

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* *Biometrika*, Vol. VII. p. 64.

† Pearson, *ibid.* Vol. I. p. 260.

We next treated, for purposes of comparison, the selected count of 15,000 cells in a similar fashion. Table VI contains the actual frequencies, Table VI A the constants and equations. Let us now consider in detail points suggested by these analytical results. The values of P show that in all cases the fit is close except in that of the sample hundreds. It appeared to us probable that in this case we had adopted too fine a unit of grouping, so that we re-worked, using a coarser unit which gave 11 groups, with the following result,

$$y = 29{\cdot}9734 \left(1 + \frac{x^2}{(4{\cdot}4732)^2}\right) \times e^{3{\cdot}7028 \tan^{-1}(x/4{\cdot}4732)},$$

which gives $P = {\cdot}232$.

This finding confirms the suggestion to some extent, but it must be admitted that the 100's are still definitely less satisfactory than the smaller samples; the explanation of this is not clear. Table VI B, which gives all the actual and calculated frequencies reduced to a common scale, will show the reader how little change was effected by the rejection of 5000 cells.

TABLE VI.

Actually Observed Frequencies of the Samples from 15,000 *Cells.*

Samples of 25		Samples of 50		Samples of 100	
Number of Bacilli per Sample	Number of Samples	Number of Bacilli per Sample	Number of Samples	Number of Bacilli per Sample	Number of Samples
44— 51	1	*112—123*	1	*267—282*	2
52— 59	11	*124—135*	8	*283—298*	8
60— 67	29	*136—147*	15	*299—314*	3
68— 75	62	*148—159*	25	*315—330*	10
76— 83	98	*160—171*	48	*331—346*	21
84— 91	115	*172—183*	66	*347—362*	27
92— 99	107	*184—195*	50	*363—378*	30
100—107	78	*196—207*	47	*379—394*	27
108—115	56	*208—219*	22	*395—410*	10
116—123	23	*220—231*	9	*411—426*	6
124—131	15	*232—243*	6	*427—442*	3
132—139	4	*244—255*	1	*443—458*	1
140—147	1	*256—267*	1	*459—474*	0
		268—279	0	*475—490*	0
		280—291	1	*491—506*	2
Totals	600	—	300	—	150

TABLE VI A.

Curves Resulting from an Analysis of 15,000 *Selected Cells.*

Material	Number of Samples	Mean (in terms of Bacilli per Sample)	Mode (in terms of Bacilli per Sample)	σ (in terms of Bacilli)	β_1	β_2
Samples of 25	600	91·1133	89·0117	16·3123	·0457	2·8211
Samples of 50	300	182·2200	179·4383	24·5984	·0980	3·7994
Samples of 100	150	363·5667	360·1649	37·0043	·1019	4·5418

Material	κ_2	r	m	ν	a	β_2-3	Skewness	P(Fit)
Samples of 25	− ·0702	—	—	—	—	− ·1789	·1288	·9966
Samples of 50	+ ·0582	12·4217	7·2109	− 3·0885	6·7230	+ ·7994	·1131	·7365
Samples of 100	+ ·0292	7·4301	2·7151	− 1·2890	5·7783	+ 1·5418	·0920	·2164

Equations:
$$y=114{\cdot}724\left(1+\frac{x}{7{\cdot}1842}\right)^{7{\cdot}0048}\left(1-\frac{x}{12{\cdot}8362}\right)^{12{\cdot}5157}.$$

Unit of grouping 8 bacilli per 25 cells. 39·5 bacilli per 25 cells taken as zero.

$$y=45{\cdot}045\left[1+\frac{x^2}{6{\cdot}7230^2}\right]^{-7{\cdot}2109}\times e^{3{\cdot}0885\tan^{-1}\frac{x}{6{\cdot}7230}}.$$

Origin at 4·7217, where 12 bacilli per 50 cells form the unit of grouping and the zero is at 105·5.

$$y=26{\cdot}445\left[1+\frac{x^2}{5{\cdot}7783^2}\right]^{-4{\cdot}7151}\times e^{1{\cdot}2890\tan^{-1}\frac{x}{5{\cdot}7783}}.$$

Origin at 5·5642, where 16 bacilli per 100 cells are the unit and the zero is at 258·5.

Next as to the agreement between the theoretical constants of the curves of means and their empirical values as above determined. Table VII gives the calculated values of B_1 and B_2-3 [assuming that where n is the number of individuals in the sample and β_1, β_2 the constants of the original distribution $B_1=\frac{\beta_1}{n}$ and $B_2-3=\frac{\beta_2-3}{n}$] together with the actual values.

In only one case, that of the 25's from the selected 15,000, is the agreement at all possible. "Student," in a very interesting communication*, has suggested that the divergence noted by him in our previous curve of 80 means, depended on the existence of homotyposis in the samples. This suggestion no doubt contains some truth and could be satisfactorily tested by mixing up our individual records of leucocytes and drawing really random samples. We have not felt justified in delaying publication of our results until we could find leisure to undertake this very laborious task, particularly as other factors are involved. This is clear from the fact that the divergence is more marked in the case of the larger samples which must be less influenced by homotyposis.

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* *Biometrika*, Vol. VII. p. 210.

TABLE VI B.

Comparison of the Observed and Calculated Frequencies for Samples of 20,000 *and* 15,000 *Cells. All the Frequencies are reduced to* 100.

Twenty-Fives				Fifties					Hundreds				
20,000 Count		15,000 Count		Bacilli	20,000 Count		15,000 Count		Bacilli	20,000 Count		15,000 Count	
Observed	Calculated	Observed	Calculated		Observed	Calculated	Observed	Calculated		Observed	Calculated	Observed	Calculated
·13	·35	·17	·22	*112—123*	·5	·5	·33	·49	*267—282*	1	1·0	1·33	1·09
1·75	1·40	1·83	1·51	*124—135*	2·25	1·54	2·67	1·61	*283—298*	4·5	1·86	5·33	1·95
4·75	4·22	4·83	4·75	*136—147*	5·25	4·17	5·0	4·49	*299—314*	2	4·52	2·0	4·49
9·25	9·47	10·33	10·74	*148—159*	7·5	9·24	8·33	9·99	*315—330*	6·5	8·97	6·67	8·91
16·0	15·71	16·33	16·20	*160—171*	14·5	15·71	16·0	16·73	*331—346*	14·5	14·03	14·0	14·79
18·12	19·44	19·17	18·89	*172—183*	20·75	19·80	22·0	20·52	*347—362*	18	17·14	18·0	18·72
18·38	18·44	17·83	17·75	*184—195*	17·75	18·63	16·67	18·65	*363—378*	17	16·68	20·0	18·03
14·13	13·89	13·0	13·73	*196—207*	16·0	13·63	15·67	13·10	*379—394*	17	13·40	18·0	13·61
9·25	8·63	9·33	8·80	*208—219*	7·75	8·23	7·33	7·52	*395—410*	7·5	9·28	6·67	8·50
4·63	4·61	3·83	4·65	*220—231*	3·75	4·36	3·0	3·75	*411—426*	5·5	5·77	4·0	4·68
2·38	2·18	2·50	2·00	*232—243*	2·5	2·13	2·0	1·71	*427—442*	2·5	3·34	2·0	2·39
·88	·95	·67	·68	*244—255*	·75	1·00	·33	·74	*443—458*	1·5	1·85	·66	1·18
·25	·39	·17	·21	*256—267*	·25	·46	·33	·50	*459—474*	·5	1·00	—	1·13
0	·15			*268—279*	·5	·39	0		*475—490*	·5	·54	—	
0	·09			*280—291*			·33		*491—506*	·1	·59	1·33	
0				*292—303*					*507—522*	·5			
·13													
100·03	99·92	99·99	100·13	—	100·00	99·79	99·99	99·80	—	100·00	99·97	99·99	99·47

TABLE VII.

Number in Sample	B_1 (empirical)	B_1 (calculated)	B_2-3 (empirical)	B_2-3 (calculated)
(20,000 Count)				
25	·1033	·0415	·4321	·0505
50	·1467	·0208	1·0907	·0252
100 (1st curve) ...	·3005	·0104	1·3501	·0126
100 (2nd curve) ...	·3142	·0104	1·4601	·0126
(15,000 Count)				
25	·0457	·0415	− ·1789	·0494
50	·0980	·0208	·7994	·0247
100	·1019	·0104	1·5418	·0124

We can now examine the accuracy of the opsonic method in the way attempted in our former paper, viz. we can inquire what the chances are of obtaining from a "population" of means, samples giving different indices in terms of the real mean of all such samples. In similar cases, it is usual to divide the frequency curve into a series of equal areas, setting up ordinates on either side of the mean or mode. Since this elaborate graphical method requires the services of a specially skilled draughtsman, and considerably increases the cost of production, we decided to adopt the plan followed in our last paper. A considerable number of additional ordinates were calculated for each sample curve and we determined the area from the beginning or end of the curve up to specified ordinates which corresponded to indices of ·6, ·7, ·8, etc. in terms of the mean. From these areas the Tables of Chances (VIII, IX, X) were deduced.

TABLE VIII.

Chances of Obtaining Certain Deviations in Index Values.

Opsonic Index in terms of the Mean	SAMPLES OF 25	
	Fraction of the Total Area bounded by the corresponding ordinate (Total Area = 1)	Odds against the occurrence of such a Deviation or a Greater
·6	·0089	111 to 1
·7	·0396	24·3 to 1
·8	·1276	6·8 to 1
·9	·2973	2·4 to 1
1·1	·2740	2·7 to 1
1·2	·1324	6·6 to 1
1·3	·0558	16·9 to 1
1·4	·0211	46·4 to 1
Beyond the limits *1·4—·6*	·0300	32·3 to 1
" " *1·3—·7*	·0954	9·5 to 1
" " *1·2—·8*	·2600	2·9 to 1
" " *1·1—·9*	·5713	·75 to 1

TABLE IX.

Chances of Obtaining Certain Deviations in Index Values.

Opsonic Index in terms of the Mean	Samples of 50	
	Fraction of the Total Area bounded by the corresponding ordinate (Total Area = 1)	Odds against the occurrence of such a Deviation or a Greater
·7	·0112	88·3 to 1
·8	·0618	15·2 to 1
·9	·2283	3·4 to 1
1·1	·2160	3·6 to 1
1·2	·0770	12 to 1
1·3	·0248	39·3 to 1
Beyond the limits *1·3—·7*	·0360	26·8 to 1
" " *1·2—·8*	·1388	6·2 to 1
" " *1·1—·9*	·4443	1·3 to 1

TABLE X.

Chances of Obtaining Certain Deviations in Index Values.

Opsonic Index in terms of the Mean	Samples of 100	
	Fraction of the Total Area bounded by the corresponding ordinate (Total Area = 1)	Odds against the occurrence of such a Deviation or a Greater
·7	·0006	1666 to 1
·8	·0216	45·4 to 1
·9	·1661	5·0 to 1
1·1	·1618	5·2 to 1
1·2	·0420	22·8 to 1
1·3	·0109	90·8 to 1
Beyond the limits *1·3—·7*	·0115	86·6 to 1
" " *1·2—·8*	·0636	14·7 to 1
" " *1·1—·9*	·3279	2·1 to 1

If we adopt about a ten to one chance as the limiting value for evidence of differentiation, then the limits, for single determinations of the index, are in the case of 25's, roughly 1·3—·7; for 50's, 1·25—·75; for 100's, 1·2—·85. Everyone has his own standard of accuracy or reliability; all we mean is that unless a given index is beyond these limits, the chances are less than ten to one against its being drawn from a normal population. We now come to a most important qualification of the value of these tables.

In the table as given, the measure is of the probable deviation of a sample from the real mean of the samples as determined from a long count. If the control mean, i.e. the denominator of the fraction which we call the opsonic index, has been determined from a large number of cells, say 1000, and the tested serum is measured on the basis of a small sample, then our results are directly applicable; but this supposition does not represent the usual state of affairs. In ordinary practice, two small samples, each of not more than 100 cells, serve as control and test, there is therefore, so to speak, a double source of variation; odds based on the results obtained above would over-estimate the reliability of any given determination. This is a point of considerable importance which has, we think, escaped the attention of writers on the subject; its elucidation would involve a careful examination of the whole theory of index distributions, and would

TABLE XI.

Constants of the Experimental Index Frequencies.

[Mean, Mode and σ are expressed in terms of the Index, i.e. in physical units.]

Material	Number of Samples	Mean	Mode	σ	β_1	β_2	κ_2	r
25's	800	1·0362	·9432	·2585	·7099	4·3914	·9619	—
50's*	400	1·0205	·9536	·2204	·6952	4·8945	·3635	11·2690
100's	200	1·0120	·9689	·1553	·3654	3·7138	·9032	—

Material	m	ν	a	$\beta_2 - 3$	Skewness	y	p	P (Fit)
25's	—	—	—	1·3914	·3600	326·1439	27·4967	·0853
50's	6·6345	− 8·5166	5·6343	1·8945	·2912	—	—	·8953
100's	—	—	—	·7138	·2780	491·3142	48·7621	·9355

Equations:

$$y = \text{antilog } 43{\cdot}6116 \left(x^{-27\cdot4967} \times e^{-\frac{326\cdot1439}{x}}\right).$$

A change of ·1 in the index is taken as the statistical unit of grouping and index ·25 as zero.

$$y = 6{\cdot}2092 \left(1 + \frac{x^2}{5{\cdot}6343^2}\right)^{-6\cdot6345} \times e^{8\cdot5166 \tan^{-1} \frac{x}{5\cdot6343}}.$$

A change of ·1 in the index is taken as the statistical unit of grouping and index ·45 as zero. The origin is at 1·4468.

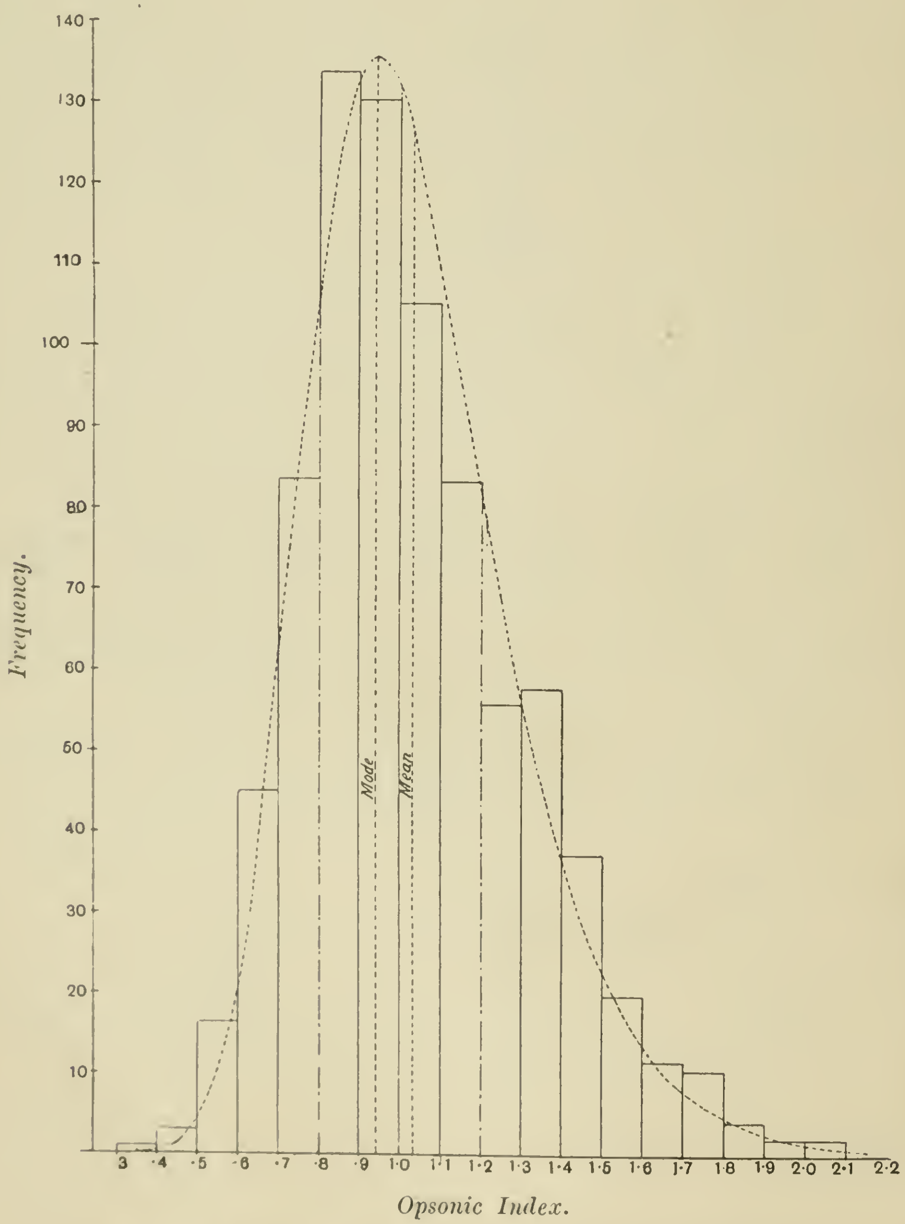
$$y = \text{antilog } 71{\cdot}8335 \left(x^{-48\cdot7621} \times e^{-\frac{491\cdot3142}{x}}\right).$$

A change of ·1 in the index is taken as the statistical unit of grouping and index ·45 as zero.

* A slight slip has been found in the values of β_1 and β_2 for this series, they should be ·6836 and 4·9855. This error does not, however, visibly alter the form of the curve in Graph 7.

accordingly present features of interest quite apart from the particular case of the opsonic index. These remarks apply with special force to medical statistics which so frequently involve the use of ratios or indices.

The importance of the problem tempted us to devote some little time to a theoretical consideration of the subject and we arrived at a few analytical results which might, we thought, be of some value. It is, however, unnecessary for us to publish these incomplete investigations, since Professor Karl Pearson has succeeded in obtaining a full solution of the problem*. We therefore confine ourselves to an empirical solution which is sufficient for our immediate object.



GRAPH 6. Curve of 25's secondary without replacement.

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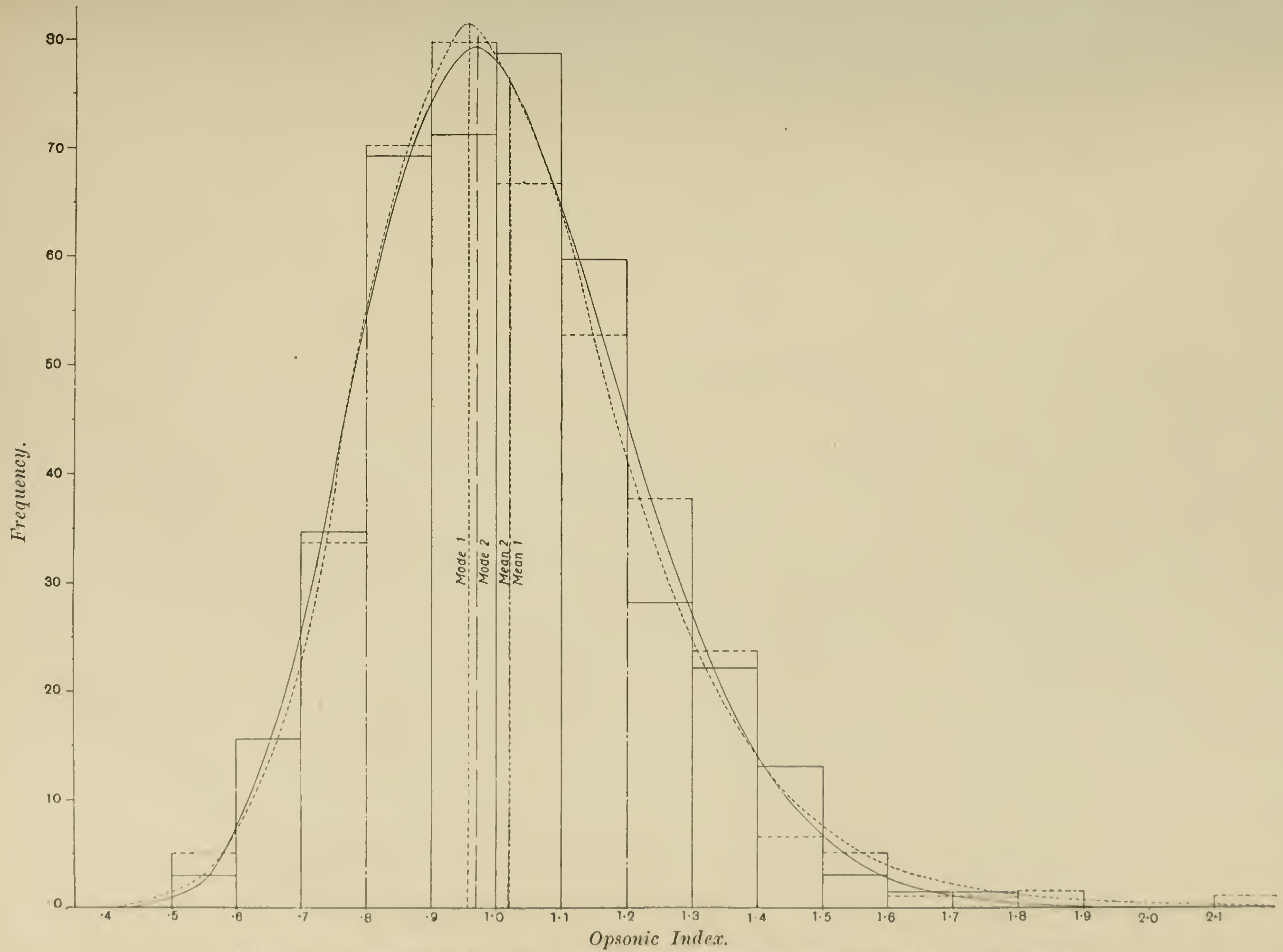
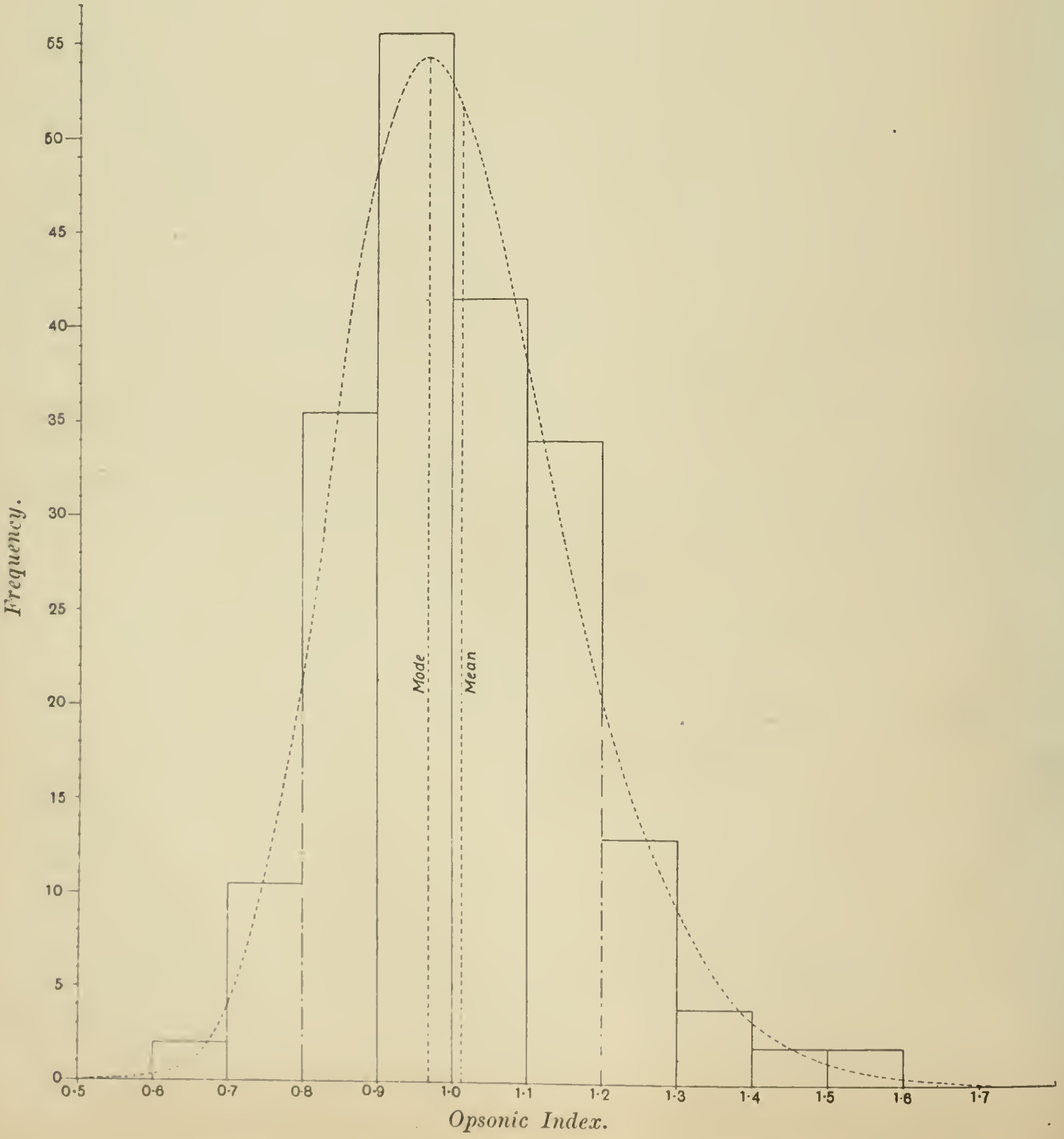
* See the following paper.



GRAPH 7. Curves of 50's with replacement—continuous: without replacement—dotted.

The actual number of bacteria in each sample of 25, 50 or 100 cells was copied out on a separate slip of cardboard, so that we had 800, 400 and 200 cards corresponding to the samples of 25, 50 and 100 cells. The cards used were small pieces of correspondence cards which, being smooth, could be shuffled very easily.

With these cards the following experiments were performed. Taking the case of the 400 cards relating to the fifties, the cards were thoroughly shuffled and one drawn out. The number on the card was written down, the card *not* being replaced. The remainder were once more shuffled, another drawing was made and the process was repeated until the whole 400 had been drawn. The cards were then once more shuffled and the process repeated, the second set of numbers being written down one by one under the first set of values. Each number of the first set was then divided by the corresponding number in the second set and the 400



Graph 8. Curve of 100's secondary without replacement.

indices thus obtained were analysed as usual. The same experiment was carried out in the case of 25's and 100's. Tables XI, XI A and Graphs 6, 7 and 8, contain the constants and data of the distributions and Table XII the chances of obtaining indices within assigned limits deduced from the areas.

TABLE XI A.

Experimental Determination of Index Distributions.

Index	Samples of 25 (Drawings not Replaced)		Samples of 50 (Drawings not Replaced)		Samples of 50 (Drawings Replaced)		Samples of 100 (Drawings not Replaced)	
	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated
·3— ·4	1	·073 } 1·527	—	—	—	—		
·4— ·5	3	1·454 }	—	—	—	—		
·5— ·6	16·5	11·243	5	3·989	3	3·007	—	
·6— ·7	45	40·468	15·5	13·990	15·5	15·308	2	1·500
·7— ·8	83·5	89·850	33·5	37·969	34·5	39·863	10·5	11·697
·8— ·9	133·5	121·939	70	66·729	69	65·497	35·5	36·009
·9—1·0	130	134·204	79·5	80·213	71	77·683	55·5	52·780
1·0—1·1	105	122·028	66·5	71·759	78·5	72·001	41·5	46·583
1·1—1·2	83	96·635	52·5	51·973	59·5	54·817	34	29·003
1·2—1·3	55·5	69·188	37·5	32·676	28	35·425	13	14·177
1·3—1·4	57·5	46·012	23·5	18·792	22	19·873	4	5·850
1·4—1·5	37	28·990	6·5	10·249	13	9·830	2	2·145
1·5—1·6	19·5	17·560	5	5·452	3	4·334	2	1·00
1·6—1·7	11·5	10·339	1	2·870	1·5	1·717		
1·7—1·8	10·5	5·967	1	1·512	1·5	·814		
1·8—1·9	4	3·397	2	·803 }	—			
1·9—2·0	2	1·918	0	·432 } 1·735	—			
2·0—2·1	2	2·0	0	·5 }	—			
2·1—2·2	—	—	1		—			
2·2—2·3	—	—	—	—	—			
Totals	800	803·265	400	399·908	400	400·169	200	203·744

A comparison of these tables with nos. VIII—X indicates how far the limits of reliability are narrowed by a more complete study of actual conditions (see also Table XV *infra*).

It will be noticed that these experiments do not assume any special distribution for the original variates. They merely afford a partial answer to this question—if we had two sets of 400—200, 800—samples exactly the same as the 400 (200, 800) actually counted and formed at random 400 (200, 800) indices from them, one set always furnishing the denominator and no sample being used more than once, how are the resulting indices likely to be distributed? In strictness, therefore, these results are only applicable to our particular case. If, however, we assume that our counts are numerous enough and accurate enough to give the distributions of sample means of the size used, and for the emulsion used, with fair exactitude, then we can perform another experiment.

TABLE XII.

Chances of Obtaining Certain Deviations on the Basis of Three Experimental Index Distributions. (Method of Non-Replacement.)

Opsonic Index	Samples of 25		Samples of 50		Samples of 100	
	Fraction of Total Area bounded by corresponding Ordinate	Odds against such a Deviation or a Greater	Fraction of Total Area bounded by corresponding Ordinate	Odds against such a Deviation or a Greater	Fraction of Total Area bounded by corresponding Ordinate	Odds against such a Deviation or a Greater
·6	·0160	61·5 to 1	·0098	101 to 1	—	—
·7	·0665	14·0 to 1	·0448	21·3 to 1	·0075	132 to 1
·8	·1727	4·79 to 1	·1397	6·16 to 1	·0659	14·2 to 1
·9	·3247	2·08 to 1	·3067	2·26 to 1	·2460	3·07 to 1
1·1	·3546	1·82 to 1	·3135	2·19 to 1	·2571	2·89 to 1
1·2	·2336	3·28 to 1	·1835	4·45 to 1	·1121	7·92 to 1
1·3	·1475	5·78 to 1	·1019	8·81 to 1	·0413	23·2 to 1
1·4	·0899	10·1 to 1	·0549	17·2 to 1	—	—
1·5	·0537	17·6 to 1	·0293	33·1 to 1	—	—
1·6	·0317	30·6 to 1	·0157	62·7 to 1	—	—
1·7	·0188	52·2 to 1	—	—	—	—
ond the Limits *1·4—·6*	·1059	8·44 to 1	·0647	14·5 to 1	—	—
„ „ *1·3—·7*	·2140	3·67 to 1	·1467	5·82 to 1	·0488	19·5 to 1
„ „ *1·2—·8*	·4063	1·46 to 1	·3232	2·09 to 1	·1780	4·62 to 1
„ „ *1·1—·9*	·6793	·47 to 1	·6202	·61 to 1	·5031	·99 to 1

Let us suppose that the distribution of samples of 50 that we obtained is actually the distribution of samples of 50 in general, then the following experiment may be considered. The 400 cards were shuffled and drawn as before except that after each drawing the card was replaced, the process being carried on until 400 numbers had been written down, a second series of 400 numbers was then obtained in the same way and the indices were computed. Tables XIII, XIV and Graph 7 give the statistical constants and other information which resulted from an analysis of this experiment. It will be noticed that the table of chances does not differ appreciably from that due to the other experiment (XV). On this account, and because of the doubtfulness of the assumption in the case of 100's and the smaller practical interest in the case of 25's, the experiment was not repeated in these cases.

TABLE XIII.

Constants of the Experimental Frequency Distribution Obtained by the Method of Replacement. (Mean, Mode and σ, Expressed in Terms of the Opsonic Index.)

Mean	Mode	σ	β_1	β_2	r	b	m_1	m_2
1·0196	·9697	·2056	·2077	3·2155	62·7551	44·6934	9·0844	51·6706

a_1	a_2	κ_2	$\beta_2 - 3$	Skewness	P
6·7337	37·9597	$-$·8536	·2155	·2428	·8988

Equation :
$$y = 80{\cdot}0808\left(1+\frac{x}{6{\cdot}7337}\right)^{9{\cdot}0844}\left(1-\frac{x}{37{\cdot}9597}\right)^{51{\cdot}6706}.$$

A change of ·1 in the index is taken as the statistical unit of grouping and index ·45 as zero.

TABLE XIV.

Chances of Obtaining Certain Deviations, on the Basis of an Experimental Curve from Samples of 50 (Method of Replacement).

Opsonic Index	Fraction of Total Area bounded by corresponding Ordinate	Odds against such a Deviation or a Greater
·6	·0075	132 to 1
·7	·0458	20·8 to 1
·8	·1454	5·88 to 1
·9	·3096	2·23 to 1
1·1	·3165	2·16 to 1
1·2	·1795	4·57 to 1
1·3	·0910	9·99 to 1
1·4	·0413	23·2 to 1
1·5	·0167	58·9 to 1
1·6	·0059	168 to 1

TABLE XV.

Comparison of Odds against Certain Index Deviations as Calculated on Different Assumptions for Samples of 50.

Index	Using True Mean	Experimental Curve without Replacement	Experimental Curve with Replacement
·7	88·3 to 1	21·3 to 1	20·8 to 1
·8	15·2 to 1	6·16 to 1	5·88 to 1
·9	3·38 to 1	2·26 to 1	2·23 to 1
1·1	3·63 to 1	2·19 to 1	2·16 to 1
1·2	12·0 to 1	4·45 to 1	4·57 to 1
1·3	39·3 to 1	8·81 to 1	9·99 to 1

Without in the least wishing to underestimate the shortcomings of both our material and method, it appears to us that Table XII can fairly be used as a rough practical test of the accuracy of the opsonic method in the case of the Tubercle Bacillus and that it confirms the opinions expressed in our former paper. A theoretical discussion of the index problem in general is reserved for another paper and more suitable material. It is now proper to consider very briefly some practical points.

In the first place, it has been asserted that the Tubercle Bacillus is an unfavourable organism to select for the purpose of studying the accuracy of the opsonic technic. While we cannot assert that no more favourable example could have been chosen, we are of opinion that the Tubercle Bacillus is certainly not the least satisfactory of the organisms commonly dealt with. For instance, Tubercle Bacilli act, or are acted on, almost entirely as single individuals, while Staphylococci or Streptococci occur in groups or chains respectively. These groups or chains are counted as so many individuals which, from the point of view of phagocytosis, may or may not be correct. The opsonic power required to attract a group of cocci to the leucocyte *may* not be greater than that associated with the ingestion of a single coccus. Under such conditions we have the difficulties as to the correct treatment of doublets and triplets—to which allusion has been made by Harvey and McKendrick—in an acute form.

Next as to the practical accuracy of the method for diagnostic purposes or in the control of dosage. The results here communicated, which are in agreement with our previous observations and also, *mutatis mutandis*, with those of Harvey and McKendrick, seem to demonstrate that single observations falling within the limits of 20 °/ₒ on either side of the mean cannot, in the case of Tubercle Bacilli, be regarded as strongly suggestive of abnormality. If it is possible, as will often be the case when it is a question of diagnosis alone, to repeat the observation several times under comparable conditions, the opsonic method may be expected to yield results of a certain value. When, however, the opsonic index is used to control injections of Tuberculin, so that no two determinations are made under the same conditions, we do not think the method has more than a very restricted field of usefulness. This leads us on to refer to a cognate matter.

Glynn and Cox use language* which seems to imply that we have attacked the opsonic index in the sense of denying that differences in phagocytic power as between normal and diseased persons occur. We have not asserted this nor does any statement of ours bear such an interpretation. We have merely pointed out, a contention not seriously challenged, that the claims advanced by certain enthusiasts to the effect that all variations of 10 °/ₒ from the normal are probably or necessarily significant, must be entirely unjustifiable.

At the risk of being tedious, we desire to emphasise once more the distinction between vaccine therapy on the one hand and opsonic estimations on the other. If our work tends, as it certainly may tend, to unsettle the opinion of some readers as to the relative accuracy of the tuberculo-opsonic index, we wish in no way to be ranked among detractors of the claim of vaccine therapy to be regarded as a new and powerful weapon in the struggle against disease. On the contrary we are convinced of its present importance and look to the future for its still greater development.

In conclusion we would again remark that the results here published are in essential agreement with those communicated by Harvey and McKendrick and ourselves in recent numbers of *Biometrika*.

Conclusions.

1. Large phagocytic counts extending to 15,000 or 20,000 cells are markedly skew and exhibit signs of heterogeneity.

2. The exclusion of clumped bacilli has not been the main cause of the poor fit obtained.

3. The heterogeneity may be due to difficulties in counting.

4. The distributions of the means of samples of 25, 50 and 100 cells drawn from these populations are also markedly skew and excellently represented by Pearson's type curves.

5. This skewness cannot be due entirely to homotyposis.

6. The odds against a second sample of the same material giving an opsonic index, in terms of the true mean, outside of the limits 1·2 to ·8 are, in the case of samples of 25, about 3 to 1, in the case of 50's about 6 to 1 and for 100's about 15 to 1.

7. When the index is estimated on the basis of two small samples and without determining the mean of the normal series from a long count, the odds are reduced to 1·5 to 1, 2 to 1 and 4·6 to 1 respectively, for samples of 25, 50 or 100.

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* Glynn and Cox: *Biochemical Journal*, Vol. IV. p. 300.

8. Even in the case of samples of 100, it is doubtful whether single determinations which give values even beyond the limits 1·3 to ·7 can be regarded as satisfactory evidence of differentiation.

9. The two previous conclusions are based on the analysis of four experimental frequencies.

10. The Tubercle Bacillus is not necessarily an unfavourable test of the opsonic method.

11. The opsonic index is less useful as a means of controlling treatment than for diagnostic purposes, since in the latter case it may be possible to make several comparable determinations.

12. Differentiation in respect of phagocytic power (Tubercle Bacilli) can possibly be established by the opsonic index in some cases.

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Recent Researches on Alcoholic Fermentation.

By Arthur Harden, F.R.S., D.Sc., Ph.D., F.I.C. (Biochemical Department, Lister Institute).

About six years ago I had the honour of reading a short communication to this Institute on the subject of zymase and alcoholic fermentation, in which I gave a short account of the work done by Buchner, and, if I recollect rightly, a practical demonstration of alcoholic fermentation by means of yeast-juice. Since the year 1904 a large amount of work has been done both in England and abroad on the subject of alcoholic fermentation, with the special object of discovering the processes which go on and how they are carried out. The work of Buchner proved a great stimulus to this line of research, and anyone looking through the literature of fermentation since 1897 will see that a large number of papers has been written and a large amount of work done as the direct result of the new means of investigation provided by his researches. I propose to give a very short account of the work done since 1904, both here and abroad, on this same question, as to the nature of alcoholic fermentation. It will be impossible to go into the matter fully in the short time at my disposal, particularly as I want to try and demonstrate to you some of the phenomena which occur. I propose to deal mainly with the work done on yeast-juice rather than on living yeast, important as the latter is.

In the first place, in 1904, I was able to indicate at this Institute and at the Royal Society the fact that Mr. Young and myself had found that the juice extracted from yeast by the grinding process contained two substances, both of which were essential for fermentation. This was shown in the first instance by dialysing the juice. It was found that when yeast-juice was subjected to rapid dialysis, something passed through the membrane and left behind an inactive residue which would no longer ferment sugar. When the portion which passed through the membrane was examined, it was found that it also would not ferment sugar, but when the two were reunited the sugar was fermented. The substance of this nature which will pass through a dialyser, and which is therefore probably comparatively simple in nature, is called—it is rather a clumsy term—the co-enzyme. The name enzyme is given to all soluble ferments, and is here restricted to the inactive material which is left behind in the dialyser. The apparatus for carrying out the dialysis consists of a Chamberland candle impregnated with gelatin and supported in a brass case, and in the annular space between the two the yeast-juice is poured. The apparatus is connected with a compressed air cylinder, and filtration is carried out under a pressure of about 50 atmospheres.

It was found that the liquid which filtered through, even after being boiled, still possessed the power of activating the residue, and that boiled and filtered yeast-juice also had the same property. During the process of fermentation both these substances gradually disappear. When yeast-juice is mixed with sugar and incubated, fermentation proceeds for a certain time, and then gradually dies out, long before the sugar is exhausted. The reason for this is that both the enzyme and the co-enzyme in the juice gradually become destroyed. Exactly what it is which destroys them is not quite certain, but the most probable view is that of Buchner, that the enzyme is gradually digested by the proteoclastic enzyme contained in the juice. What destroys the co-enzyme is a much debated question; it does not appear to be the proteoclastic enzyme which destroys the enzyme itself, but rather something of the nature of a lipase, an enzyme which decomposes fats, liberating glycerol and fatty acids. This is interesting, because it is the only clue so far to the chemical nature of the co-enzyme, which, since it is decomposed by lipase and not by proteoclastic enzymes, appears to be allied rather to the fats than to the proteïns. It was further found that these two substances disappear at unequal rates. We found in the first instance that if we added only a small quantity of the co-enzyme to a solution containing sugar and the enzyme deprived of the co-enzyme, only a very limited fermentation occurred. That showed that the co-enzyme under the prevailing conditions died out before the enzyme. Buchner has made an interesting observation on this point; working with German bottom yeasts, he obtains a juice in which the co-enzyme regularly dies out before the enzyme. There is a deficiency of this co-enzyme, so that when he incubates the yeast-juice with sugar the fermentation comes to an end because the co-enzyme has disappeared. But if he then adds a further quantity of co-enzyme he gets renewed fermentation, and can prolong a fermentation, which would ordinarily end in three days, for almost three weeks by repeatedly adding fresh quantities of the co-enzyme as soon as fermentation ceases. This shows that in the yeast-juice obtained by Buchner the enzyme is capable of being preserved for a long time, provided it is in the presence of a sufficient quantity of co-enzyme. With English top-yeasts that is not the case. When the fermentation comes to an end it cannot again be revived by adding the co-enzyme; both the enzyme and the co-enzyme apparently die out at approximately the same time in the juice we get from English yeast. This is the first point established as to the nature of the fermentation: that there are these two different agents concerned in its production, and that either of them alone is quite inactive, but that when the two are mixed fermentation proceeds. As to exactly what is going on and what is the precise nature of either the enzyme or the co-enzyme we know very little. That is one of the subjects which has so far eluded everybody's skill. Nobody has yet got hold of a pure enzyme and found out what sort of a substance it is, and the same is true so far of the co-enzyme. We have been quite unable, as Buchner has been unable, to isolate it and ascertain what sort of substance it is.

In the second place we found that there was another agent present which had a great effect in stimulating fermentation, and by making use of a special form of apparatus we were able to analyse this effect. The fermenting mixture is put into a small flask kept at a constant temperature of 25° C. in a thermostat. This flask is connected by a tap with an apparatus for collecting the gas—the ordinary apparatus which is used for collecting nitrogen. The gas which is given off is CO_2, which is easily soluble in the fermenting liquid, and the solubility of course varies with the pressure. In order to get over errors due to this cause we keep the pressure constant, and in order to do that we fit the mercury reservoir with a syphon, so that all the mercury which passes into the reservoir syphons out and runs over the edge of a small glass cylinder, so that the level always remains the same, and the pressure in the flask is always that of the atmosphere. The volume of gas in the nitrometer is then read off at a diminished pressure and corrected for the difference of pressure by means of a table. We found that when we took yeast-juice and put it into an apparatus like this, and then added a boiled solution of the co-enzyme with which we had been working, there was a sudden rush of gas quite soon after the mixture had been made—in the first 15 or 20 minutes after adding the boiled yeast-juice or the filtrate obtained in the experiment I have previously described. We set to work to try to account for this phenomenon, and after trying a number of different materials, finally hit upon the fact that it was due to the presence of phosphates. We could reproduce this effect by adding any one of the soluble phosphates, sodium phosphate or potassium phosphate, and produce an effect quite similar to this sudden increase in the rate of evolution of gas which occurred when that filtrate was added. We found, in the first place, that there was evidently a definite chemical reaction going on; that the phosphate was not acting as the enzyme did, but as a chemical substance acts; because the carbonic acid produced was exactly equivalent to the phosphate which was added. If we added a molecular proportion of phosphate we got off, in addition to what would otherwise have been produced, a molecular proportion of carbonic acid. Further experiments have shown, in the first place, that when phosphate is added the rate of fermentation is greatly increased. This increase varies in different cases from 10 to 20 times the rate at which the original fermentation was going on. Secondly, this increase in the rate only lasts until the chemical equivalent of carbon dioxide has come off, and then the rate falls again to what it was in the original experiment before the phosphate was added. If a second addition of phosphate be made, precisely the same phenomena occur again, and this can be continued until the volume of the liquid or the concentration of salts becomes too great.

The next point was to find out what had become of the sodium phosphate. We boiled the yeast-juice which had been used for such an experiment, tested it with magnesia mixture to estimate the phosphorus, and found that the latter was no longer in a form which could be precipitated by magnesia mixture; it was no longer present in the form of a mineral phosphate at all, but in some other form. We were then able to isolate from the juice to which the phosphate had been added an entirely new compound, which is a compound of a hexose residue and phosphoric acid, to which we have given the name of hexosephosphate. This substance forms an insoluble lead salt which, when treated with sulphuretted hydrogen, gives an acid, salts of which have been prepared and analysed by my colleague, Mr. Young. There is a discussion going on at the present moment as to the exact structure of this substance. A little later than ourselves the Russian chemist, Iwanoff, found evidence of the presence of this substance in yeast-juice which had been fermented. And since then another Russian chemist, named Lebedew, has also examined this substance. We consider that the hexose is combined with two molecules of phosphate and that the hexosephosphate has the formula $C_6H_{10}O_4(PO_4H_2)_2$. Lebedew, on the other hand, thinks there is only one molecule of phosphoric acid in the ester. The reason for this wide divergence of opinion is that the salts are all amorphous; it has not been possible to crystallise any of them and obtain a definite crystalline salt which can be subjected to analysis. Our analyses of the amorphous salts, however, all agree with the formula given above. Lebedew has not himself analysed the salts, but from the composition of the phenylhydrazine derivative has come to the conclusion that the ester contains only one molecule of phosphoric acid. Assuming for the moment that our view is right, we have come to the following explanation:—When the phosphate is added a molecule of sugar is broken down into carbonic acid and alcohol, and another molecule of sugar combines with the phosphate and forms this hexosephosphate. Whether those are two distinct molecules or whether two fragments derived from different sugar molecules unite and form this hexosephosphate has not been decided. At all events, the equation, as far as we can make out, shows that two molecules of sugar are concerned, and that an amount of alcohol and carbonic acid corresponding to one of these is given off. The reaction, which only proceeds in the presence of enzyme and co-enzyme, is represented by the following equation:—

$$2C_6H_{12}O_6 + 2Na_2HPO_4 = 2CO_2 + 2C_2H_6O + 2H_2O + C_6H_{10}O_4(PO_4Na_2)_2.$$

The production of this substance, hexosephosphate, is very remarkable, because it does not seem to be, strictly speaking, an intermediate product. It appears that the hexosephosphate is not made first, and then decomposed by subsequent fermentation, so as to serve as the sole source of the carbonic acid and alcohol. On the contrary, it is made in the greatest quantity and at the greatest rate at the moment when another portion of sugar is breaking down into carbonic acid and alcohol. That leads us to favour the view that very probably what happens is, that the two molecules of sugar are broken into two parts, and that one part of each molecule is converted into carbonic acid and alcohol, and the other two parts reunite and form this derivative, the hexosephosphate. A certain degree of confirmation of this idea is given by the fact that, when this hexosephosphate is prepared from glucose, we find that on boiling it with acid we do not get glucose back again, but we get lævulose, if not entirely, at all events in large part. Exactly the same thing happens when mannose is used; a hexosephosphate is formed which, when boiled with acid, yields a large proportion of lævulose. It is evidently not a very simple change that occurs.

When an excess of phosphate is added, a phenomenon occurs which differs to some extent from the one I have been describing. As the amount of phosphate is gradually increased, the foregoing phenomena are gradually obscured by a secondary effect. This may be illustrated by the effect of adding four different quantities of phosphate—5, 10, 15, and 20 c.c.—to the same quantity of yeast-juice, making the liquid in each case up to the same volume. In the first case, an immediate effect is produced. Ten minutes after the addition of 5 c.c. of phosphate, the rate has risen from 1·5, the original rate, up to 13. At the end of about a quarter of an hour the rate has become 17, about twelve times as high as that of the original yeast-juice. The supply of phosphate then becomes exhausted by conversion into hexosephosphate, and a rapid fall occurs down to the original rate. When 10 c.c. are added the rise is delayed; again there is the equivalent given off at an enhanced rate, and then again a rapid fall. When 15 c.c. are added, another effect becomes evident; the phosphate exerts some sort of deleterious effect on the enzyme, and the rate rises very slowly and never gets up to the true maximum, and again falls very slowly. With 20 c.c. that effect is exaggerated; and if a still higher concentration of phosphate were used, there would be no increase at all, or even a diminution. The exact cause of this has not been ascertained, but the reason why I have described this phenomenon is that it has brought out a very marked difference between lævulose and glucose. If experiments of this kind were made with lævulose instead of glucose, it would be found that in the presence of lævulose a far greater amount of phosphate could be added without producing this effect. In the presence of lævulose the enzyme is very much less susceptible to phosphate, and at the same time fermentation proceeds at a much greater rate—three or four times as quickly as with glucose. With lævulose and phosphate we have obtained a rate with yeast-juice which is as much as half the rate which could be produced by the living yeast from which the juice was obtained. The rate of fermentation by yeast-juice alone is only about one-fortieth of the rate at which the yeast from which the juice was prepared would ferment sugar, so that by the addition of lævulose and phosphate we have been able to increase the rate from one-fortieth to about one-half, an observation of great interest in its bearing on what is going on inside the yeast-cell.

We are now in a position to discuss the cause of the difference between this high rate in the presence of added phosphate and the low rate in the absence of added phosphate, which constitutes the normal rate of fermentation of yeast-juice. As already mentioned, the hexosephosphate is not a true intermediate product; nevertheless it is broken down in the juice, and converted back again into some sort of sugar and free phosphate. Both of these, together with a fresh quantity of sugar, enter into reaction, and the whole process is repeated; so that the phosphate goes through a continuous cycle. First phosphate, next hexosephosphate, then back again to phosphate, and then again to hexosephosphate. What we imagine is, that in the ordinary yeast-juice, in the absence of added phosphate, the rate at which the fermentation goes on is entirely controlled by the rate at which the phosphate is produced. We have found that this action is due to a second enzyme, which we have called, following the ordinary rule of adding *-ase* to the name of the substance decomposed, by the somewhat barbarous term *hexosephosphatase*. This enzyme brings about the reaction according to the following equation:

$$C_6H_{10}O_4(PO_4Na_2)_2 + 2H_2O = C_6H_{12}O_6 + 2Na_2HPO_4,$$

and the rate at which this reaction proceeds determines the normal rate of fermentation in yeast-juice. When phosphate is added, a measure is obtained of the amount of the actual enzyme present; because then the enzyme is supplied with as much phosphate and sugar as it can deal with, and consequently a greatly increased rate is produced.

We were naturally led to try a number of other substances as well as phosphate, to see whether the action was peculiar to phosphate. One of the first tried was one chemically very similar to a phosphate, namely, an arsenate. We tried to ascertain whether sodium arsenate would have the same effect as phosphate. As a matter of fact, it has been found that the rate is very considerably increased by the addition of arsenate. One very marked difference, however, exists, namely, that the arsenate does not seem to act at all in the same way as the phosphate; there is no definite equivalent of carbonic acid evolved. The evolution goes on for a long time after the equivalent is produced; and many times the equivalent is produced at the high rate, so that you do not have the characteristic falling off in the rate that you find with phosphate. We have been recently examining this matter, and we have arrived at some results which are not yet published in detail; but, without giving figures, I may say we have found that arsenate does not take the place chemically of phosphate. We tried an experiment by getting rid of practically all the phosphate in the juice, and, instead of adding phosphate, we added arsenate to see whether this reaction would go on. It did not go on; and we have ultimately come to the conclusion that arsenate acts in quite a different way from phosphate by stimulating the action of the hexosephosphatase. We have also directly proved that, in the presence of arsenate, hexosephosphate is very much more rapidly decomposed than in yeast-juice alone without the presence of arsenate; and this confirms the conclusion that the way in which the arsenate acts is by stimulating the hexosephosphatase.

It appears probable that in the living yeast-cell we have the phosphate going through this cycle just as it does in the yeast-juice; and that the main difference between fermentation by living yeast and by yeast-juice is the rate at which the phosphorus goes through this cycle. From the amount of phosphorus contained in yeast and the rate of fermentation, we can calculate the number of times that the phosphorus of the yeast must go through this cycle in order to produce the observed rate of fermentation. This comes out at about twice in five minutes at 25° C.; and therefore, if the reaction goes on in that way, in living yeast it is necessary for the whole of the phosphorus to go through the cycle twice in every five minutes. The best imitation we have of what is going on in the yeast-cell seems to be this mixture, sugar and yeast-juice with a little arsenate, fermenting steadily at a rate about 20 times as great as that which the yeast-juice alone produces. It is not intended to imply that in the yeast-cell there is arsenate; but simply that the high rate of action of the hexosephosphatase is common to the cell and the mixture of juice and arsenate. This leads us to ask: What is it that actually does occur; is there some substance in the yeast-cell which has this stimulating effect on the hexosephosphatase which we can imitate by the action of arsenate; or is it due to some definite arrangement—I will say for want of a better word—inside the yeast-cell? There is no doubt that in every living cell there is a very complicated structure; and there is also no doubt that when this structure is destroyed the chemical action of the cell is very considerably modified. Professor Armstrong and E. F. Armstrong have recently pointed out the importance of some very interesting phenomena of this kind—the action of anæsthetics on leaves. It is found that when leaves containing glucosides which yield hydrocyanic acid are exposed to an anæsthetic, there is an immediate production of hydrocyanic acid. That is evidence that the anæsthetic upsets the balance of forces within the cell, whether physically, by affecting the water present, or by destroying the structure of the cell, is an open question; but at all events it does bring about some sort of disorganisation which causes a chemical action to begin which is not going on normally. I have found a very similar effect with yeast; when it is exposed to toluene the rate of auto-fermentation is very greatly increased. When yeast is kept, it auto-ferments at the expense, presumably, of glycogen; but when exposed to toluene the rate is greatly increased, four or five times. This is probably due to some change in the constitution of the yeast-cell, and indicates at all events that a very slight disorganisation of the cell is sufficient to produce a marked change in the chemical reactions proceeding within it. It is significant that yeast itself does not respond to sodium phosphate in the same way as does yeast-juice. Living yeast (this was first found by Dr. Slator, and was confirmed by myself) does not respond when treated with phosphate, and the rate of fermentation is scarcely affected. If anything is done to destroy or to upset the organisation of the yeast cell—if the yeast be dried and heated, or ground, or treated with alcohol or with acetone, in every case a material is obtained which at once responds to phosphates to a greater or less degree. The most responsive material is obtained by the complete destruction of the cell. When the yeast is ground and the liquid pressed out, a juice is obtained which responds most easily to phosphate. If the yeast be treated with acetone or ether, it does not respond to phosphate nearly so strongly, the rate being only doubled or trebled, whereas in the case of yeast-juice it is multiplied 20 times. This seems to indicate that in the yeast-cell there is something in the nature of an arrangement inside the cell which conditions the very high rate of activity of the yeast as compared with the juice prepared from it.

There is only one other subject I want to touch on for a moment, which is of very great theoretical interest, and conceivably, although not very probably, may be of practical interest. That is, the question of the exact chemical change through which the sugar passes in alcoholic fermentation. At the time I read the paper in 1904 there had been just started by Buchner a theory that in the formation of alcohol and carbonic acid from sugar, lactic acid was an important intermediate product. He was of opinion that all the sugar went through the form of lactic acid before it was converted into carbonic acid and alcohol. Since that day, largely due to criticism by Dr. Slator, Buchner has been induced to repeat his experiments, and to carry out new ones; and he has finally and officially abjured the belief that lactic acid is an intermediate product in the formation of carbonic acid and alcohol from sugar. This theory has in the meantime taken possession of practically the whole of botany and physiology; and there will be a good deal of reconsideration required now that lactic acid is no longer to be adhered to. However, a new favourite has arisen instead of lactic acid, in the form of dihydroxyacetone, $CH_2(OH){\cdot}CO{\cdot}CH_2(OH)$, which is derived from glycerol by oxidation.

Buchner has found that dihydroxyacetone has a much greater claim to the position of an intermediate product in sugar fermentation than lactic acid, because he has found that living yeast will ferment it, although only slowly; and also that yeast-juice, under certain conditions, will bring about its fermentation. However, the matter is not quite straightforward; because, if dihydroxyacetone be added to yeast-juice, the fermentation is extremely slow, and it is necessary to add a large volume of boiled yeast-juice containing the co-enzyme, phosphates, and many other things, before vigorous action occurs. It is conceivable that the production of carbonic acid and alcohol, which Buchner ascribes to the fermentation of dihydroxyacetone, may really be due to some secondary effect, or to the conversion of this substance, by condensation, into a fermentable compound. This point cannot be considered settled till many more experiments have been carried out.

The production of a three-carbon substance from sugar as an intermediate product has been a very favourite theory for a long time. It seems unlikely that a large molecule like that of glucose should be split up without undergoing several stages, and this is also the result of the action of alkalis on glucose. Further, we have always to bear in mind that a compound containing three carbon atoms, namely, glycerol, is always formed during fermentation. Glycerol is formed, not only by living yeast, but also by yeast-juice; so that it seems most likely, although that again is not quite certain, that it is actually formed from the sugar. If a substance like dihydroxyacetone really occurs as an intermediate product, it affords an obvious opportunity for the production of glycerol by reduction; and this idea is, therefore, so far consistent with the fact that we do actually get a three-carbon compound in all fermentation of glucose by yeast. It is also quite consistent with the production of hexosephosphate, because it may be that there is an intermediate production of some three-carbon compound, and that the hexosephosphate is formed in the way previously suggested.

If we sum up the position of affairs so far, it is that ever since the discovery by Buchner of yeast-juice, matters have been getting constantly more and more complicated. It has been found that the yeast-cell does not simply contain an alcoholic enzyme which can be compared with a substance like invertase, but that it is also provided with a regular army of enzymes and co-enzymes; that fermentation involves the presence of phosphate; that with each of these enzymes there is provided another enzyme which gradually destroys it. Moreover, Buchner has recently established the fact, which was indicated in my last paper here, that the cell also contains a substance which protects the enzyme from the proteoclastic enzyme.

It is obvious, therefore, that the problem of alcoholic fermentation, which has exerted so great a fascination over workers at all periods in the history of science, is still far from complete solution, and offers as many points of interest for the future as have rewarded the investigators of the past.

The effects of the addition of phosphates and arsenates to yeast-juice and sugar were demonstrated experimentally.

Harrison and Sons, Printers in Ordinary to His Late Majesty St. Martin's Lane.

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ON THE ALLEGED PRESENCE OF AN ALCOHOLIC ENZYME IN ANIMAL TISSUES AND ORGANS.

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THE nature of the processes involved in the utilisation of carbohydrate material by the animal organism has always been obscure, and, despite the voluminous literature on the subject, our present position amounts to little more than a recognition of the obvious fact that sugar is easily and abundantly destroyed in the healthy body.

The isolation of a glycolytic enzyme from yeast by Buchner suggested that a similar enzyme might be present in animal tissues; according to this view glucose was supposed to be split up in the tissue cells into alcohol and CO_2, the alcohol in turn being oxidised into CO_2 and H_2O. This hypothesis seemed to be substantiated by actual experiment, and though certain objections were raised from time to time with regard to the experimental evidence, the occurrence in the tissues of an enzyme capable of bringing about a true alcoholic fermentation of sugar has steadily gained ground; at the present time its presence seems to be fairly generally accepted.

The evidence adduced in favour of this view is:

1. When the organs of dead animals are incubated in contact with sugar, alcohol and CO_2 are formed; the same result is obtained by using tissue juices or powders prepared from such juices by precipitation with alcohol and ether.

2. Traces of alcohol, or of some body reacting as such, are found in the fresh organs and in the blood of all vertebrate animals investigated. In certain cases it seems almost certain that the substance obtained was actually alcohol.

The question of the presence or absence of a glycolytic ferment is obviously of such fundamental importance in all work having as its basis the investigation of carbohydrate metabolism in animals, that no advance in this direction seems probable until this point is settled.

The results of the present investigation show that all the evidence hitherto brought forward is capable of explanation without the necessity of assuming the presence of a zymase-like ferment in the tissues; in fact, it is certain that much of this evidence rests on a very unstable foundation, and has been wrongly interpreted.

Observations on the disappearance of sugar in animal tissues or juices have been made by Claude Bernard(1). Lepine(2), Kraus(3), Spitzer(4), Arthus(5), Lauder Brunton(6) and many others. These workers, however, discussed principally the destruction of sugar, but did not deal with the mechanism involved.

Among the first to suggest the presence of a zymase-like enzyme in animal tissues was Blumenthal(7). He found that expressed juice of pancreas was capable of destroying glucose with the formation of CO_2, but failed to detect alcohol. In 1903, however, Stoklasa(8) stated that the process of sugar destruction by the animal tissue was essentially of the same nature as the fermentation of sugar by yeast, and even claimed to have succeeded in isolating the active substance. The correctness of the statement has been questioned by many observers who were unable to obtain Stoklasa's results. Mazé(9) found that the alcohol-ether precipitate from ox lung gave a vigorous fermentation when mixed with glucose, but bacteria were always present; this reaction was not a true alcoholic fermentation, for, although alcohol and CO_2 were formed, a good deal of hydrogen was evolved, and considerable quantities of lactic acid were also detected. Somewhat similar negative evidence was adduced by Batelli(10) and by Portier(11). In spite of these criticisms, however, Stoklasa has continued to advocate the correctness of his views with such persistence that their acceptance seems to be gradually gaining ground. It may be at once admitted that the experiments, described by Stoklasa and his colleagues, have been carried out with great care, and also that, under the conditions employed, a destruction of sugar takes place with the formation, among other products, of carbon dioxide and alcohol. The only question arising is whether the precautions adopted were sufficient to eliminate the possibility of bacterial invasion. Stoklasa and his colleagues claim to have proved the absence of bacteria, but it will be shown later that the methods employed were quite unsuited for the purpose.

The theory that lactic acid forms an intermediate product in the process of alcoholic fermentation has undoubtedly exercised a considerable degree of influence on the development of the experimental work carried out with animal organs. Both Stoklasa and Ransom(12) regard the well-known production of lactic acid in animal tissues after death as a step in the process of alcoholic fermentation. Stoklasa, in fact, finds that large amounts of lactic acid are produced in his experiments; this is accounted for by supposing that, in these cases, the acid is being formed from sugar by the zymase more rapidly than it is being decomposed by the so-called lactacidase. It has, however, now been shown by Slator(13), and also by Buchner and Meisenheimer(14), that there is no experimental evidence in favour of the view that lactic acid does actually form an intermediate stage in the conversion of sugar into alcohol and carbon dioxide, and, until some fresh evidence is adduced, this cannot be admitted. It seems probable, therefore, that the post-mortem formation of lactic acid in animal tissues is not in any way connected with alcoholic fermentation, and, moreover, that the formation of lactic acid in glucose solutions in presence of animal tissues, juices, and powders is also quite independent of the presence of an alcoholic enzyme in these materials.

Details of method by which juices and powders were obtained. In the literature concerning the subject of this paper there is such a marked tendency to explain certain divergent results as dependent on minor differences of manipulation, that it is necessary to give exact details as to the methods utilised in procuring the substances used. In all experiments with powders and juices, except those in which some deviation is specially described, the following procedure was adopted.

The material was obtained as soon as possible after death, either from an animal which had been killed in the laboratory, or from the slaughter-house; in the latter case the organ was kept at a low temperature during transit by means of ice. It was then minced as finely as possible, and the mass mixed thoroughly with an equal amount of silver sand. This mixture was thoroughly ground up in a large mortar, not more than 300 gms. to 400 gms. being used each time. The disintegrating effect of the sand caused the formation of a soft, sticky, pasty mass. To this was added kieselguhr in amount sufficient, when thoroughly mixed, to render the whole fairly consistent. This was again thoroughly ground up as before, until it also became somewhat pasty. Finally, sufficient kieselguhr was added to form a mass sufficiently firm and dry to form a coarse powder when rubbed between the hands. The juice was obtained from this mass by means of a hydraulic press capable of exerting a maximum pressure of over three tons per square inch. The expressed juice was collected in a tall glass cylinder surrounded by ice. The whole process was carried out as quickly as possible, and, in general, the juice was obtained in from 1½ to 3 hours after the death of the animal; in some cases special efforts were made to complete the process within 1 hour after death.

From this juice the powder was prepared as follows: 50 c.c. of juice were added slowly and with constant stirring to a mixture consisting of 400 c.c. absolute alcohol and 200 c.c. ether. This was at once thrown on to a large filter connected with a suction pump. By this means the precipitate could generally be separated from the alcohol-ether mixture in from 1½ to 2½ minutes. The residue was then washed with ether, spread out on a filter paper, and dried in vacuo either at room temperature or at 35° C. In some cases it was simply spread out in a thin film, and left exposed to the air of the laboratory. The whole mass dried very quickly whatever process was adopted, and in from five to fifteen minutes formed a powder which crumbled on being pressed. After being thoroughly dried, the whole was ground in a mortar to a very fine powder, and in this form used for the experiments.

In a few of our experiments we obtained the tissue-juices and powders according to Stoklasa's slight modification of Buchner's method: this consists in waiting till the precipitate separates out from the alcohol-ether mixture, and settles at the bottom of the vessel. The alcohol is then decanted off, and the precipitate thrown on to a filter, and washed with ether. As it was found that direct filtration with the help of a suction pump was the more expeditious method, this plan was adopted in the great majority of the experiments.

Since Buchner has pointed out that alcohol exercises a deleterious effect on zymase, and that in precipitating the ferment by means of alcohol it is necessary to work very quickly in order to limit as much as possible this destructive tendency, a comparative experiment was made with yeast-juice. A sample of active yeast-juice was taken and 50 c.c. added slowly and with constant stirring to a mixture composed of alcohol 400 c.c. + ether 200 c.c. This was at once poured on to a large filter, and the residue washed with ether. The time between the instant at which the first portion of juice came into contact with the alcohol and the end of the washing with ether was exactly three minutes. The precipitate obtained was then dried in vacuo and ground into a fine powder; when quite dry it weighed 4·4 gms. The activity of this powder was estimated at 37° against 50 c.c. of the same juice under similar conditions as follows:

Exp. 1.	Amount of CO_2 given off in 5 hours
5 gms. dextrose + 50 c.c. juice (Sat. at 37° with CO_2)	86·2 c.c.
5 gms. dextrose + 50 c.c. H_2O + 4·4 gms. powder (Sat. at 37° with CO_2)	82

From these figures it is clear that alcohol does not appreciably reduce the fermenting activity of zymase when the contact is not longer than three minutes. Similar results have been obtained by Buchner.

According to Stoklasa it is of very great importance that the tissue juice should be expressed at a pressure of 300 to 400 atmospheres; he makes the statement that powders obtained from juices expressed at pressures below 250 atmospheres are relatively inactive, while a pressure of 300 to 400 atmospheres yields a fluid which furnishes a very active powder.

Unfortunately, the meaning of the statement that his active juices were obtained at a pressure of 300 to 450 atmospheres is not very clear; if this means that the press gauge registers this pressure, it conveys no information whatever, for the pressure actually exerted on the tissue may be either greater or less than this in proportion to the relative sizes of the plates used. In Buchner's press the actual pressure exerted per square inch is much less than that indicated by the gauge, and, as Stoklasa seems to use one of these presses, it is obvious that the actual pressure on the material at which he obtains his juice is really considerably less than 350 to 400 atmospheres. Since, however, he considers the question of pressure to be of maximum importance, it was thought advisable in our experiments to state the exact pressure at which the juice was obtained; this in every case refers to the actual pressure exerted on the tissue, and not that registered by the gauge.

Method of estimating alcoholic fermentation. The purpose of this investigation being to find out whether or not alcoholic fermentation in tissues was an established fact, no experiments have been made to ascertain any variations in the actual amount of sugar present before and after the experiment; the possibility of sugar being destroyed in some other way than by conversion into CO_2 and alcohol is not discussed. As an index of fermentation we have measured the amount of CO_2 given off in any given time. This was done by means of the apparatus fully described by Harden, Thompson and Young(15), in which the flask containing the fermenting material is connected with an azotometer, into which the gas passes over as it forms. After making allowances for pressure in the ordinary way, the volume of gas given off in any given period can be accurately determined. The instrument is exceedingly delicate, and well suited for the determination of a small amount of fermentation. It, moreover, possesses the great advantage of providing an accurate index of what is actually taking place at any given time in the course of the experiment, and so furnishes most valuable information, which it is quite impossible to obtain when the CO_2 is collected in KOH and weighed as done by Stoklasa, and this after long intervals. All our experiments were carried out at a temperature of 37° C., the procedure being as follows.

A certain amount of aqueous solution of dextrose was warmed to 37° in the water bath of the apparatus; it was then connected with the azotometer and saturated with CO_2; when equilibrium was established the powder or other substance was added, and the whole again saturated with CO_2. By removing the flask from the bath at short intervals and shaking vigorously, any CO_2 that tended to remain in the flask in a supersaturated condition was driven off, so that equilibrium at 37° was soon established. Any trace of CO_2 now generated by the mixture at once increased the pressure, and a corresponding amount of gas passed over into the azotometer. In this way an accurate index of what was happening inside the fermenting flask could be obtained without disconnecting or interfering in any way with the apparatus. The nature of the gas given off is, of course, impossible to ascertain in cases where only traces are evolved, for, since the whole apparatus is filled with CO_2 to begin with, a corresponding amount of this gas is displaced, whatever be the nature of the gas generated. This, however, applies only to small quantities, for, after a short time, if the fermentation is at all vigorous, the actual gas generated passes over. It is advisable in all cases to saturate the mixture with CO_2 at the beginning of the experiment, otherwise some of the gas formed at first may be held in solution in the fluid, and its presence overlooked; this precaution also ensures anærobic conditions.

Discussion of results previously obtained with powders prepared from tissue juices. An examination of the results obtained by the advocates of alcoholic fermentation shows that the amount of CO_2 evolved is relatively very great. Thus Stoklasa obtained the following figures in certain of his experiments using 5—10 gms. of material.

Powder from flesh in 18 hours = 0·732 gms. CO_2.
,, ,, lungs ,, 24 ,, = 1·72 ,,
,, ,, liver ,, 60 ,, = 0·7621 ,,
,, ,, flesh ,, 52 ,, = 1·38 ,,

And somewhat similar results have been obtained by Simácek(16) and others. Nearly all the experiments, however, suffer from the drawback that observations were recorded only after the sugar and powder mixture had been incubating for many hours. If an alcoholic ferment is really present, it ought to exert its maximum action in a short time, and then gradually decrease in efficiency. The possibility of the presence of a pro-enzyme will be discussed later.

Observations taken at short intervals are of much greater value than those taken for instance every 12 hours, for the former give some indication whether the rate of reaction is at all in accordance with what might be expected from bacterial action.

Experiments without antiseptics. It is generally admitted that antiseptics have a marked influence in inhibiting the action of tissue preparations when incubated with sugar, and Stoklasa and his followers explain this as a detrimental action of the antiseptics on the alcoholic enzyme. Simácek, recognising this action, did not use any antiseptics in some parts of his work, but relied on a highly concentrated sugar solution to exclude bacterial action. Oppenheimer(17), who seems to accept Stoklasa's views, observes that the antiseptics used with a view to eliminate the action of "bacteria" may have been applied in such concentration as to have defeated their purpose, in that they caused destruction of the enzyme; he thus explains the results of those observers who have been unable to confirm Stoklasa's work. On reading Oppenheimer's observations the impression is obtained that this author treats the cry of "bacteria" as something which has been much overdone; he makes the statement that bacteria do not account for the results obtained by Stoklasa and his colleagues. Unfortunately, the evidence on which this statement is based is wanting. Stoklasa adduces but little proof that the fermentations observed by him have taken place shortly after the mixtures were placed in the incubator. It is true that in a few instances it is said that "fermentation began immediately," but in many cases this would seem to refer to what *appeared* to be fermentation, the appearance of some bubbles or froth in the mixture. (In two cases, however, it is claimed that after two hours 0·272 gm. and 0·23 gm. CO_2 were found.) In many of our experiments we have observed this effect, but it was in no case accompanied by evolution of gas, and appeared to be caused by the manipulation necessary to mix the sugar solution and powder. In appearance it often closely represents what is seen in an ordinary yeast-juice fermentation, and, if appearance alone were considered, it might certainly be taken as evidence of fermentation. Our apparatus, however, gave us the great advantage of knowing exactly whether or not this frothing during the first few hours coincided with the production of gas, and our observations prove that it is simply a physical phenomenon, and connected in no way with fermentation. It is very likely that some of Stoklasa's results are to be explained in this way. In no single case have we been able to obtain any real evidence of fermentation for some hours after the mixture had been incubated at 37°.

Action of tissue-juice powders on glucose solution. The general result obtained is seen in Exp. 2. After mixing the materials, it takes about ½ hour to get equilibrium established, and after this it will be noticed that little or no gas is given off for about 5½ hours, when fermentation begins. In some cases a very small trace of gas is evolved during the first few hours, but it will be shown later that this is dependent on physical conditions, and does not indicate fermentation. It is always slight in amount, and would in no case be of any importance when considered in relation to the large amounts of CO_2 claimed to have been observed by the advocates of alcoholic fermentation.

Exp. 2. 15 gms. ox liver powder, obtained from juice prepared at a pressure of 3 tons per square inch of material, were mixed with 50 c.c. of 10 % dextrose solution which had been previously saturated with CO_2 at 37° C. The mixture was again saturated with CO_2, and placed in the water bath at 37° at 11.25 a.m. It was twice shaken, and the first reading taken at 11.30: records were then taken every half-hour for 12 hours.

Time after commencement of exp., in hours	Amount of gas given off in c.c.	Observations	Time after commencement of exp., in hours	Amount of gas given off in c.c.	Observations
0	2·2	—	6·5	1·8	—
0·5	1·5	Many bubbles and slight froth.	7	·6	—
1	—		7·5	1·2	—
1·5	—	—	8	1·5	—
2	1·1	—	8·5	2·6	—
2·5	—	Slight froth as if fermenting.	9	6·3	Froth very marked.
3	—		9·5	14·0	—
3·5	—	—	10	26·3	—
4	—	Distinct froth.	10·5	49·8	—
4·5	—	—	11	50·2	—
5	—	—	11·5	56·0	—
5·5	—	—	12	54·2	Flask full of froth.
6	3·8	—			

In this experiment only 4·8 c.c. of gas were given off in the first 5½ hours, and of this amount 3·7 c.c. were evolved shortly after the mixture was made as the result of establishment of equilibrium. This leaves the small amount of 1·1 c.c. gas in 5½ hours as possibly due to alcoholic fermentation. That this is not its origin will be shown by later experiments. After this, fermentation went on slowly but steadily, and increased every ½ hour, in many cases doubling itself in this time. It attained a maximum after 11½ hours, and continued for a considerable time, gradually diminishing in intensity during the next 12 hours, till, ultimately, in three to four days, the action ceased. This experiment is a good example of what is obtained in all similarly conducted experiments; naturally, there are certain unimportant variations as regards amount of gas given off and other particulars, but the general picture is always the same, and shows that fermentation begins only after several hours. This can only mean that, if the fermentation is due to an enzyme, the substance was originally present in the form of a zymogen; on the other hand, it points very strongly to bacterial infection. It is well known that many bacteria, when growing in suitable media, can double their numbers in about ½ hour; this observation agrees fairly well with what is observed by experiment, there being a marked tendency for the amount of gas evolved during the first few hours of commencing fermentation to double in amount every half hour. The possibility of a zymogen, however, was investigated, and, as it was noticed that fermentation was always accompanied by the production of lactic acid, some powder was incubated with a small amount of this acid in the presence of sugar; it was thought possible that the acid might act as an activator to the zymogen, if present. The result, however, was always the same; fermentation began as usual after some hours, and pursued the same course as before. All experiments in this direction agreed in furnishing no evidence whatever of the presence of any zymogen-like body. On the other hand, *bacteria were found in every case.*

In these experiments powders obtained from the following tissues and organs were used:

Ox = Liver, kidneys, pancreas, blood, lungs, heart.
Rabbit = Flesh, liver.
Guinea-pig = Flesh.
Pig = Liver and pancreas.

In no case was any evidence of an alcoholic enzyme obtained. In certain cases modifications of the above-described processes were tried, but always with the same result, viz.: that fermentation began after a certain number of hours in all cases, but bacteria were then always present. In many of our experiments elaborate precautions were adopted to prevent bacterial invasion in the hope of obtaining an aseptic substance, but the results have convinced us that it is practically impossible to obtain an aseptic powder, whatever precautions are adopted. It might be thought that treatment with alcohol would destroy any bacteria present, but it must be remembered that in the preparation of the powder the contact is of such short duration as to render it very doubtful whether this is perfectly effective in this respect. Even if it were so, it is extremely difficult, if not impossible, to prevent subsequent infection during the drying of the powder. The extreme difficulty, or, probably, impossibility of obtaining an aseptic powder can only be realised by those who have actually tried the experiment. Often the cases in which the most elaborate precautions were adopted yielded powders which seemed to be more infected than others obtained without adopting any special precautions. The following experiment is interesting in showing that bacteria may escape the action of alcohol.

Exp. 3. A sample of fermenting guinea-pig flesh was found to contain in abundance a long, rather thick bacillus. A broth consisting of peptone and extract of bullock's heart, to which a little sodium chloride had been added, was inoculated with this bacillus; about 2 °/₀ dextrose was also added, together with some calcium carbonate, and the whole put into the incubator at 37° for 30 hours. The solution was then treated with alcohol and ether, exactly as the tissue juices. A small amount of a precipitate was obtained, the whole manipulation taking 4½ mins. This powder was carefully dried in vacuo at 35°, and added to a mixture consisting of 25 c.c. of the above broth containing 1 °/₀ dextrose. The flask was then placed in the water bath at 37°, saturated with CO_2, and the result observed, allowance being made for establishment of equilibrium.

Time, in hours	No. of c.c. gas given off	Time, in hours	No. of c.c. gas given off
0	—	5·5	0·1
1	—	6·75	—
3·25	—	7·5	—
3·75	—	9·5	5·2
4	0·3	10·25	12·7
4·5	0·8	10·75	9·2
5	—	11·25	11·0

Demonstration of bacteria in the fermenting mixture. The method adopted was to take a small amount of the fermenting material on a slide, and form a thin film, which was dried, and fixed by means of heat in the usual way. It was then placed in an alcoholic methylene blue solution for a minute or two, and examined. This simple process we found very suitable. Occasionally treatment of the film with a solution of 80 °/₀ alcohol containing 1–2 °/₀ of acetic acid helped to render the bacteria evident. As a general rule no difficulty was experienced in finding them; in other cases it was a laborious process, owing to the large amount of extraneous matter on the slide, though even in these latter cases a suitable film was found to be absolutely swarming with them.

Action on glucose of the bacteria isolated from the fermenting mixture. The next point for consideration was whether the bacteria really caused the fermentation. This was tested by inoculating a suitable mixture containing sugar, which had been previously sterilised in the autoclave, with a portion of the fermenting mixture from one of the experiments. A positive result was generally obtained, though the process was not so vigorous as the original fermentation. This can be easily explained by the fact that the tissue powders provide a more suitable medium for the bacteria than does peptone. The experiment was carried out exactly as the original one, so that the conditions in both cases were identical; it was always found that the bacteria caused fermentation of sugar if *the inoculation sample was procured during the height of active fermentation.* As a rule the original fermentation of the powder continued for three to four days, and then died out; in many cases, an inoculation after this period gave no fermentation in the glucose medium. In such a case, however, the bacteria were probably killed by the acid formed as the result of fermentation, so that no growth could be expected. Bacteriologists have repeatedly shown that certain sugar-fermenting bacteria are killed by the action of their fermentation products, so that, while inoculations taken from a mixture at the beginning of fermentation show a good growth and ferment glucose vigorously, no action is obtained if the sample is taken from a fermentation that has been going on for some days(18). It is thus obvious that the failure to obtain living bacteria from a mixture that has been fermenting for some days is no proof whatever that bacteria have not been present. In several such cases the examination of films demonstrated their existence. Now Stoklasa assumes the absence of bacteria from the fact that he has been unable to obtain growth when suitable media are inoculated with the fermenting substance after the fermentation is complete, and it will be seen from the foregoing that no growth could have been expected under the circumstances. Sometimes we have obtained growth after several days of fermentation, but in other cases in which positive results were obtained at an earlier period—say after 12 hours' fermentation—no growth could be obtained after five days.

Exp. 4. Powder was obtained from the flesh of guinea-pigs in the usual way, a pressure of 2 tons per square inch having been used. 4·9 gms. powder + 50 c.c. 15 % glucose incubated at 37° gave the following figures:

Time after commencement of exp.		Amount of gas in c.c. given off since last reading	Time after commencement of exp.		Amount of gas in c.c. given off since last reading
hrs.	min.		hrs.	min.	
0	10	1·1	3	45	—
0	30	0·5	4	0	—
0	45	0·2	4	15	—
1	0	0·7	5	0	0·1
1	15	0·3	5	15	0·1
1	30	0·6	5	30	—
2	15	0·3	6	15	0·2
2	30	0·2	6	30	0·1
2	45	0·1	11	15	5·9
3	0	0·2	11	30	1·0
3	15	0·2	22	0	178·6 *
3	30	—	26	0	—

* Much gas was lost during night.

Bacteria found in abundance: many rod-like bodies.

Inoculated 28 c.c. broth containing 1 $^0/_0$ sugar incubated at 37° and observed the fermentation.

0	0	—	20	0	0·3
4	0	—	20	45	0·1
18	0	19·4	24	30	1·9

Bacteria found in fair abundance: many rod-like bodies as in original fermentation.

Exp. 5. 10 gms. ox liver powder (from juice obtained at pressure of 2½—3 tons) +50 c.c. 5 $^0/_0$ glucose sol. not saturated with CO_2.

Time in hrs. and min. after beginning experiment	No. of c.c. since last reading	
0 hrs. 0 min.	—	
0 ,, 30 ,,	0·2	
1 ,, 30 ,,	—	
4 ,, 0 ,,	—	
6 ,, 0 ,,	—	
10 ,, 15 ,,	110+	Bacteria present in abundance.

Inoculation into glucose gave 29 c.c. gas in 18 hours.

Numbers of similar experiments were carried out, and always with the same general result.

Simácek(16) states that bacterial action was prevented in his experiments by the use of highly concentrated sugar solution, but this view is not in accordance with experimental evidence. This experimenter used maltose and lactose in 30 $^0/_0$ solution, and obtained a production of carbon dioxide, which he claimed to be formed by an enzyme, but did not use dextrose in this high concentration. On testing this point, we have found that certain bacteria can grow quite well in solutions of maltose and lactose equal in strength to those used by Simácek.

Even in 30 % dextrose solution a fair amount of growth was obtained, though it was not nearly so marked as in the case of the disaccharides. This is probably due to the fact that solutions of dextrose have nearly twice as great an osmotic pressure as solutions of the disaccharides containing the same percentage of the sugar. That bacteria can flourish in 20 % solution of glucose is seen by Exp. 4, in which this concentration of sugar was employed. That 15 % dextrose solution is easily decomposed by bacteria is shown by Exp. 6, carried out with a pure culture of *B. lactis ærogenes.*

Exp. 6. A flask containing 50 c.c. of a mixture of dextrose 15 gms. + peptone 1 gm. + distilled water 100 c.c. was inoculated with the *Bacillus lactis ærogenes.* After 21 hours it yielded 22·5 c.c. gas, and continued to ferment for 26 hours, yielding an average of 2·9 c.c. to 2·3 c.c. per hour. The experiment was then discontinued.

In the light of the above evidence the presence of a zymase can only be maintained on the assumption that the enzyme is present in an inactive form and that it manifests its presence at the same time as the bacteria become active; in this way its effect would be obscured by the bacterial action, both processes going on together. The fact that activity is never manifested unless bacteria are present in numbers capable of being easily demonstrated by the microscope renders it impossible to prove such an opinion by experiments of this kind. If a zymogen were present, it is conceivable that, in some instances, its action might be obscured by bacterial contamination, but on the other hand, since bacteria take from five to eight hours or more to produce their effect, its action should sometimes be manifested earlier. That it has never once been so in a long series of experiments may be accepted as a proof that it is not present in amount sufficient to produce the results claimed for it. That traces of some such ferment may be contained in the tissues we do not pretend to deny, but, up to the present, no cogent evidence has been adduced in support of such a contention.

That a very marked amount of fermentation takes place generally within 12 hours, and that this fermentation is due to bacteria, we consider as proved. Examination of the nature of the gas given off furnishes further evidence in support of this.

Nature of gas given off in fermentation experiments. Unlike yeast fermentation, the gas given off from tissue powders is not pure CO_2. A certain amount of this gas is present, but the quantity is variable; it is always associated with an inflammable gas which is probably hydrogen. In five different samples examined the proportions of CO_2 to inflammable gas (taken as hydrogen) were as follows:

(*The figures refer to parts per hundred.*)

No.	CO_2 in c.c.	H in c.c.
1	53·8	46·2
2	28·1	71·9
3	20·3	79·7
4	43·4	56·6
5	31·5	68·5

This fact alone is very suggestive of bacterial action, although Stoklasa regards the hydrogen, which he observed in many instances, as due to enzymic action. It may be noted, however, that no independent evidence for the production of hydrogen by the action of an enzyme exists, so that this question is on exactly the same footing as that of the existence of an alcoholic enzyme in the tissues.

Experiments with Antiseptics. The chief antiseptic used was toluene; this substance, when present in suitable amount, possesses the advantage of being fairly effective in preventing bacterial growth, while, at the same time, it does not appreciably diminish the action of yeast zymase. Thus, it possesses the characteristics most suitable for the detection of a possible alcoholic ferment in the tissues. Our results with small amounts of antiseptics were similar in kind to those obtained when no antiseptics were employed, the only difference being the manifestation of a tendency for fermentation to be delayed; often it was also less vigorous. This delay was accentuated in proportion to the concentration of antiseptic used, till, ultimately, no fermentation whatever took place. Quantities of toluene that were quite ineffective in inhibiting yeast-juice fermentation absolutely prevented every trace of reaction when tissue products were used. This suggests that, if any alcoholic enzyme really exists in the tissues, it differs materially from the yeast enzyme.

It might be argued that the enzyme present in the tissues could hardly be expected to approach in amount or activity that of the yeast enzyme; such a supposition is hardly in harmony with Stoklasa's experimental results, for, if we accept the products observed by him as the results of true enzyme activity, then their comparatively large amount proves that any enzyme capable of generating them must be quite as active as that obtained from many samples of yeast-juice.

A reference to the experiments of Arnheim and Rosenbaum(19) shows that in many cases the amount of antiseptic used did not prevent bacterial action; in these cases the results were discarded. The fact that bacteria grew freely in certain cases under the same conditions as those used for the other experiments shows that the antiseptics employed were not reliable.

Many experiments were carried out by us with different amounts of antiseptic, and different results were obtained according to the amount used, as explained above; the important point, however, is, that in every case in which fermentation took place, micro-organisms, capable of fermenting sugar, were detected in abundance in the fermenting fluid. To this there was no exception; usually they were easily demonstrated; in other cases with difficulty, as already explained. In all, their presence was sufficient to account for the reaction observed.

The experiments recorded below bring out the above points.

Exp. 7. Ox liver powder prepared from juice at pressure of 3 tons was mixed with sugar solution, and different amounts of toluene added; each mixture was then incubated at 37° C. in the usual way. The effect of the antiseptic is well seen.

	Mixture used	Amount of toluene added	C.c. gas evolved	Remarks
1.	10 gms. powder + 50 c.c. 5 % glucose solution	None	113 + in 24 hours	Very vigorous fermentation. Bacteria found in abundance.
2.	,, ,, ,,	0·2 c.c. = (·4 %)	110 + in 24 hours	Very vigorous fermentation; swarming with bacteria: rate of fermentation at end of 24 hrs. was 3 c.c. gas every 5 min.
3.	,, ,, ,,	0·3 c.c. = (·6 %)	37 in 48 hours	Bacteria found; many cocci; smells strongly of toluene.
4.	,, ,, ,,	0·7 c.c. = (1·4 %)	None	—

Here 0·4 % toluene had little effect, while ·6 % caused considerable delay in fermentation. This experiment was stopped after 48 hours, for, from our experience of these mixtures, we knew that once fermentation gets a start it goes on vigorously in spite of toluene. All the above were thoroughly shaken at frequent intervals. When the flasks were left to stand without shaking, large quantities of toluene were required to prevent fermentation. In Stoklasa's method, in which hydrogen is continuously passed through the flask during the experiment, there must be a great tendency for all volatile antiseptics to be carried over.

Exp. 8. 10 gms. ox liver powder (pressure 2½ tons) + 50 c.c. 15 % glucose + 1 % toluene incubated as above gave the following figures:

In 24 hours	=	2·5 c.c. gas.
,, 48 ,,	=	71·4 ,,

After this time a very vigorous fermentation was taking place as indicated by the figures.

49. 1st hour = 11·2 c.c. gas.
50. 2nd ,, = 11·3 ,,
51. 3rd ,, = 12·8 ,,
52. 4th hour = 12 c.c. gas.
53. 5th ,, = 11·4 ,,
54. 6th ,, = 10·1 ,,

This continued for several hours and then slowly decreased in amount. It was still fermenting with some vigour three days afterwards.

Many more experiments of the same kind were made, but the results were always somewhat similar. In general, however, no fermentation was induced when more than 1 °/₀ toluene was present. In some cases less than 1 °/₀ completely prevented fermentation, at least for one or two days.

It would seem that the exhibition of a quantity of toluene, which might fairly be assumed to act as a preventive to bacterial growth, effectually prevents fermentation. Bacteria and fermentation always go together; these bacteria, moreover, are actually capable of inducing fermentation yielding alcohol and carbon dioxide among the products. There is, therefore, no reason to assume that bacteria are not the cause of the phenomenon observed. Oppenheimer's conception that bacteria only form alcohol from glucose in trifling amount is not a valid one; in reality, considerable amounts of this substance are produced by many bacteria. The following figures are interesting in this connection(31):

Organism	Sugar used	Amount of CO_2 found in percentage of sugar decomposed	Amount of alcohol formed in percentage of sugar decomposed
B. lactis ærogenes	Glucose	38·0	17·0
B. coli communis	,,	18·09	12·85
,, ,,	,,	12·1	10·2
,, ,, abnormal strain	,,	12·85	8·9
,, ,,	,,	14·6	14·33
,, ,,	,,	18·5	24·5
,, ,,	Levulose	13·07	11·5

Some of these numbers show as close a correspondence to alcoholic fermentation as many of the experiments of Stoklasa.

It is now known that toluene is a much more powerful antiseptic than thymol, and since Stoklasa in his experiments with antiseptics generally used thymol, and that in a concentration of ·4 °/₀ only, it is unlikely that its presence was of much importance in preventing bacterial growth. On the other hand Buchner has shown that thymol is more harmful than toluene towards zymase.

A curious point, difficult to explain except on the assumption of bacterial action, is encountered when we examine certain work on the action of the pancreas. Arnheim and Rosenbaum(19) found that when 1 to 2 gms. of acetone pancreas powder were incubated with

10 % dextrose solution and chloroform a considerable amount of CO_2 was given off. In three different experiments 0·058 gm., 0·140 gm., and 0·158 gm. of CO_2 were given off in 24 hours. In another case no less than 0·305 gm. CO_2 was evolved in 48 hours. Simácek found that 0·164 gms. of pancreas powder (alcoholic precipitate) gave 0·42 gm. CO_2 in four days. Stoklasa also in 1904 found that pancreas was very active. In one of his latest publications (Sep. 1909), however, he shows that pancreas powder does not contain any alcoholic enzyme, and is incapable of forming CO_2 from dextrose(20). One of these experiments is as follows:

6·2 gms. pancreas alcohol-ether powder + 50 c.c. 10 % glucose at 37°.

After 8 hours = 0·006 gms. CO_2 evolved.
0·002 ,, found in sol.
0·008 gms. together.

If this last statement is correct, then it follows that all the other observers who found the pancreas active must have been mistaken; it also proves that Stoklasa's former results with pancreas powder were not due to an enzyme; if so, we may assume that they were caused by bacteria.

In this later paper he admits that in all the cases examined bacteria were found in the fermenting mixture after 24 hours. He also states that when antiseptics were employed in the usual amount they were capable of destroying any sugar-fermenting enzyme present. This practically amounts to an admission that antiseptics are more harmful to the enzyme than to bacteria. If this is true for the pancreas enzyme, may it not with all fairness be supposed to apply to any similar enzyme in other tissues?

The acceptation of such a view throws out of court nearly all the experiments hitherto done on the subject; it is unnecessary, however, to press the argument to this conclusion; sufficient has been said to show the nature of the experimental evidence on which the existence of this enzyme is based.

Experiments with finely-minced organs and flesh. The amount of mechanical manipulation necessary to procure tissue-juice powders obviously increases the difficulties of working aseptically. It was thought that contamination might be avoided by using the tissue itself either whole or in a finely divided form. In this case mechanical interference was reduced to a minimum, and little opportunity for sepsis seemed possible. Our first experiments were carried out with minced flesh, but, in spite of the greatest precautions, it was found quite impossible to avoid infection with bacteria. Some experiments were done without antiseptics, and in others toluene was employed; the effect of antiseptics was somewhat similar to that described above, when tissue powders were used. In the case of minced tissue, however, more toluene could generally be added without inhibiting fermentation. This probably depended on the large bulk of protein present preventing thorough access of the toluene to all parts of the mass; part of the toluene also may have combined with the protein. The results obtained are seen in the next experiments.

Exp. 9. A rabbit was killed, skinned, and eviscerated: the head was cut off, and the remainder of the body passed through a mincing machine. All the usual precautions to avoid infection were taken, every vessel and instrument used in the experiment having been sterilised beforehand in the autoclave. Three portions of 40 gms. each of the minced mass were weighed in a sterile dish, and each portion transferred to a separate flask containing 60 c.c. of sterile 2 $^0/_0$ glucose. The flasks were ready twenty minutes after death.

Flask A contained no antiseptic.
,, B ,, ½ c.c. toluene.
,, C ,, 1 ,,

The contents of each flask were then saturated with CO_2 at 37°, and connected with the azotometer as usual. Readings were commenced 1 hour after the flasks were put into the bath, and continued at frequent intervals afterwards. The rate of fermentation was as follows; each reading refers to the amount of gas given off since the last reading:

Time in hours	No. of c.c. gas given off since last reading Flask A (no antiseptic)	Flask B (½ c.c. toluene)	Flask C (1 c.c. toluene)
1	—	—	—
2	0·9	1·3	1·7
5	0·6	0·2	0·4
6½	—	—	—
8½	48·7	—	—
9	20·4	—	—
9¼	39·0	—	—
9½	51·0	—	—
9¾	59·2	—	—
10¼	121·4*	2·1	2·3
22	—	22·2	2·6
30	—	24·2	14·3
48	—	100·0†	126·7‡

* Stopped. Bacteria present in great abundance. Inoculated and gave fermentation.
† Stopped. Bacteria found. Smells of toluene. Inoculated and gave fermentation.
‡ Bacteria found. Smells of toluene. Inoculated and got marked fermentation.

Exp. 10. Guinea-pigs were treated exactly as in Exp. 9. Two portions of 40 gms. each of the mixed material were added to 60 c.c. of a sterile 5 °/₀ dextrose solution. No. 1 flask contained no antiseptic, while ½ c.c. toluene was added to No. 2.

In 7 hours No. 1 evolved 56·9 c.c. gas.
,, ,, No. 2 ,, 7·2 ,,

During the next 2½ hours No. 1 evolved 109·8 c.c. gas.
,, ,, ,, No. 2 ,, 28·2 ,,

After 10½ hours No. 1 was evolving gas at the rate of 8·6 c.c. every 15 min.; No. 2 gave 8·2 c.c. in the same time. The experiment was then stopped, and bacteria found in both cases. Inoculations gave marked fermentations.

In another case an ox liver was placed in 2 °/₀ lysol as soon as possible after death, and left there for 1½ hours; it was then put into a large volume of boiling water and boiled for 2 minutes. This coagulated the protein on the outside of the mass to the depth of about ½ inch. Small portions were now removed from the centre with a sterile knife, minced with the same precautions as before, and incubated with 5 °/₀ glucose solution. In 8 to 9 hours a vigorous fermentation had taken place, and bacteria were present in great abundance. Other attempts were carried out with kidneys and flesh on similar lines, but the results were equally bad, and it seemed quite impossible to mince an organ without infecting it.

Afterwards the mincing machine was discarded, and the following experiment tried.

Exp. 11. A liver was treated with 2 °/₀ lysol for 1 hour, and then placed in boiling water for 2½ min. Small pieces were then cut out from the centre and placed directly in a 5 °/₀ glucose solution. In 6 hours vigorous fermentation was in evidence, and bacteria were present in great abundance.

Kidney treated in the same way, and boiled for 1½ min. was equally unsatisfactory, and it became evident that slaughter-house organs at any rate were useless for our purpose.

These experiments prove that the organs used by Stoklasa in cases where he incubated the whole or large parts, must have been capable of producing bacterial growth.

Our results point to the probability that it is useless to endeavour to obtain asepsis when even a very small amount of exposure and manipulation is necessary; the usual precautions adopted in such work are evidently quite insufficient to procure the desired end.

Some of our experiments were conducted so carefully that it seems impossible to account for the growth obtained by external infection during manipulation. In connection with this, it is interesting to note that Wolbach and Saiki(21) have pointed out that an organism capable of fermenting carbohydrates exists normally in the liver of healthy dogs. This organism does not grow on ordinary culture media, and cannot be demonstrated by inoculation into peptone. This fact furnishes another proof that negative inoculation experiments are not in themselves sufficient evidence that bacteria are not present. The existence of a similar organism in some of the tissues utilised may help to explain a certain number of our results. Other organisms also must have been present.

Experiments with whole organs. Stoklasa has performed various experiments with whole organs with results showing that these organs give quite an active fermentation when incubated with sugar at suitable temperature. It seemed clear that by such a method it ought to be possible to expose the organs to such a slight extent as to avoid infection. If this were possible, it would be of very great importance in settling the question.

In a few cases we have succeeded in obtaining organs free from sepsis, and in these cases there was practically *no formation of* CO_2 *or other gas* after incubation at 37° for a very long time. In the majority of our attempts, however, bacteria were present, and a reference to the data given in the following experiments shows the extreme difficulty of obtaining an organ sterile.

The experiments were carried out on rabbits. In some cases no fermentation was in evidence for periods up to 50 hours; whenever a reaction occurred, bacteria were found. If an enzyme had been present, it had ample opportunity to manifest its action during these long intervals, but in no case was there the slightest evidence of any effect of this kind.

The next experiment indicates that, where there are no bacteria, there is no fermentation.

Exp. 12. The bench on which the rabbit was dissected was thoroughly washed with 2 $^0/_0$ lysol: the same precaution was taken with regard to all glass plates and supports in the neighbourhood of the experiment. The flask in which fermentation took place was specially fitted with a thick rubber cork: through this a long bent tube passed. Into this flask was put 150 c.c. of a 5 $^0/_0$ glucose solution, and the whole sterilised in the autoclave for 1 hour, the end of the tube being plugged with cotton-wool. All the glass and rubber connections of the azotometer were similarly treated. In order to reduce the chances of infection, no saturation with CO_2 was attempted. The actual amount of gas produced is, therefore, somewhat greater than the numbers found, for a certain amount remains in solution.

A rabbit was now killed, and the hair over the abdominal area cut off; this part was thoroughly singed with a large Bunsen flame, and finally treated with a large hot iron.

The skin was cut through with a sterile knife, retracted, and the exposed surface again thoroughly treated with the hot iron. A fresh knife and forceps were now taken, and the liver exposed; the presenting part was grasped with a forceps which had just been heated almost to redness, and its connections severed, the gall bladder being snipped off. It was removed from the body as quickly as possible, and dropped into the sterilised sugar solution in the flask. The time between the exposure of the liver and its deposition in the sugar solution was only a few seconds. The whole was then incubated at 37°.

Date	Time	Gas given off in c.c. since last reading
6. 8. 10	2.15 p.m.	0·8
	2.30 ,,	0·8
	3.10 ,,	0·6
	4.0 ,,	2·7
	4.15 ,,	3·5
	6.0 ,,	—
	10.0 ,,	—
7. 8. 10	2.30 ,,	4·2
8. 8. 10	10.30 a.m.	—
9. 8. 10	10.0 ,,	—
10. 8. 10	10.0 ,,	—
11. 8. 10	10.0 ,,	—
12. 8. 10	10.0 ,,	—
13. 8. 10	10.0 ,,	Smells quite fresh after 7 days; like beef tea. No bacteria present.

Here the total amount of gas in seven days was only 11·8 c.c.; as it is well known that fresh organs always give off a certain amount of gas, which can also be obtained by boiling them with dilute acid; this small amount can be easily accounted for. At any rate it is so small as to be unimportant. This experiment of itself affords very strong evidence that an aseptic organ is incapable of producing fermentation.

Other attempts carried out with the same precautions gave several times a well-marked bacterial growth in eight to ten hours. Indeed, it was only occasionally that an aseptic liver could be obtained, and on two occasions only were we completely successful.

In the next experiment the result of incubating different organs is given; the attempt was in this case only partially successful, but it is obvious that any ferment present had sufficient opportunity to exert its action before bacterial growth took place. The organs chosen were (1) liver, (2) heart and kidneys, (3) lungs; the temperature being 37° C. The liver showed signs of bacterial growth after 51 hours; the heart and kidneys were aseptic, no growth having taken place in five days; while the lungs began to ferment bacterially after 30 hours.

Exp. 13.

	Amount of gas given off in c.c. since last readings		
Time in hours	Liver in 100 c.c. 5 % glucose sol.	Kidneys+heart in 100 c.c. 5 % glucose sol.	Lung in 100 c.c. 5 % glucose sol.
2	1·2	2·4	0·8
4	—	—	—
5	0·5	0·4	—
6	0·1	—	—
8	3·3	0·8	—
10	—	—	0·3
12	—	—	—
20	—	—	—
30	—	—	1·1
51	1·2 Began to ferment; bacteria found	—	Vigorous fermentation soon began; bacteria found
120	—	No bacteria	—
Total	6·3 c.c. gas in 51 hrs.	3·6 c.c. gas in 120 hrs.	2·2 c.c. gas in 30 hrs.

These experiments we consider as amongst the most important in the investigation, since one or two positive experiments of this kind outweigh entirely all other evidence.

Experiments with pancreas-juice powders. As above mentioned, Stoklasa(20) now claims to have proved that the pancreas does not possess a ferment capable of fermenting glucose. On the other hand, he makes the extraordinary assertion that a ferment is present which has the power of acting on disaccharides, and forming, among other products, carbon dioxide and alcohol.

So far as is known, pancreas contains no ferment capable of converting cane-sugar or lactose into glucose. Simácek, however, found that pancreas easily fermented cane-sugar and lactose; the preliminary conversion of disaccharides into monosaccharides being naturally assumed, the question arose: how does the pancreas do this, since it contains no lactase, and, probably, no invertase? The fact that fermentation was obtained, however, suggests that the whole effect was due to bacterial contamination.

Stoklasa's new observations completely changed the whole complexion of this problem, but, unfortunately, we have been quite unable to substantiate them. The juice used for preparing the powders was obtained at a pressure of one to three tons in each case.

Exp. 14. Ox pancreas was obtained as fresh as possible, and powder prepared. Two flasks made up as follows were incubated with potassium phosphate, so as to have a mixture similar to that used by Stoklasa. No saturation with CO_2.

A. 6·2 gms. powder + 50 c.c. 10 $^0/_0$ glucose + 0·5 gm. K_3PO_4.
B. ,, ,, + ,, ,, cane sugar + 0·5 gm. K_3PO_4.

In six hours A gave off 0·7 c.c. gas, and B 1 c.c. gas.

Fermentation then began, and bacteria were found in the mixture in very great abundance.

Exp. 15. (Ox liver.)

A. 4·3 gms. powder + 50 c.c. 10 $^0/_0$ cane sugar gave in 6 hrs. = 0·3 c.c. gas.
B. 3 ,, ,, ,, ,, ,, = 0·2 ,,

Exp. 16. Powder from fresh pancreas of pig prepared as before, and three flasks incubated as follows:

A. 6·2 gms. powder + 50 c.c. 10 $^0/_0$ glucose + 0·5 gm. K_3PO_4.
B. ,, ,, + ,, ,, lactose + ,, ,,
C. ,, ,, + ,, ,, maltose + ,, ,,

Previously to the addition of the powder the contents of the flasks were saturated with CO_2; the whole was then re-saturated at 37°. Readings were taken after ½ hour, when equilibrium was established. The experiment was commenced at 12.5 p.m.

	No. of c.c. gas given off since last reading		
Time	A. Glucose	B. Lactose	C. Maltose
12.30	---	—	—
12.40	1·5	0·6	1·1
1.0	0·8	0·2	0·5
1.15	0·8	1·1	—
1.20	0·2	1·0	0·7
1.30	0·7	—	0·5
1.45	0·3	—	—
2.30	—	—	0·3
3.0	0·4	—	0·8
4.0	0·9	1·1	0·5
5.0	0·9	—	—
6.0	—	—	0·8
Total in 6 hrs.	6·5 c.c.	4·0 c.c.	5·2 c.c.

During the next two hours fermentation was quite marked in each, and bacteria were found in abundance.

It will be seen that a small quantity of gas was given off by each in six hours. This was no greater with one sugar than with another. This small preliminary yield, as already indicated, depends on physical conditions, and has nothing to do with fermentation.

Several other experiments were carried out, but no evidence whatever of the presence of any enzyme acting on disaccharides or monosaccharides was obtained.

On the small preliminary yield of gas obtained. In many of our experiments with tissue powders small amounts of gas (amounting generally to a few c.c. in five or six hours) were obtained when a preliminary saturation with CO_2 was made use of. At first it was thought that this might indicate a slight fermentation, but experiment proved that such is not the case. The chief points against it being a true fermentation are:

1. It takes place when antiseptics are present in concentration sufficient to inhibit an enzyme.
2. The presence of sugar makes no difference.
3. Exactly similar results are obtained with substances which certainly contain no enzyme, *e.g.* Witte's peptone.

The phenomenon is probably to a great extent dependent on changes in the liquid, due to gradual solution of the powder. At first the liquid containing the powder is saturated with CO_2, but gradually, as more powder goes into solution, the power of the solvent to hold CO_2 changes somewhat, and small amounts of gas are given off from time to time.

Experiments in an atmosphere of hydrogen. Exception might be taken to the above experiments on the ground that anærobic conditions were procured by means of an atmosphere of CO_2. Stoklasa and his co-workers invariably used hydrogen, and it might be argued that our results are dependent on the gas used. Such a supposition is *a priori* not likely to be correct, for it has been already shown that the gas actually evolved is a mixture of hydrogen and CO_2.

In order to remove all possible objections, we carried out several experiments in an atmosphere of hydrogen, the apparatus used being arranged on the same principle as that described by Stoklasa. The CO_2 formed was absorbed by KOH in the usual way and weighed. The powder and sugar solution were incubated for about six hours, and the amount of CO_2 given off during this time was determined; in several cases, the quantity generated between the sixth and tenth hours of incubation was also estimated.

A reference to the experiments shows that traces only of CO_2 were obtained during the first six hours, but that quite appreciable amounts were evolved after nine to ten hours. As usual this was found to be accompanied by the development of bacteria. The results agree well with those obtained with a CO_2 atmosphere. The small amounts obtained in 8 hours (0·8–2·6 c.c.) are probably due to the commencement of bacterial action. They are decidedly smaller than the amounts obtained in the first 5½–6½ hours in the experiments in CO_2, in which, as explained above, a small evolution, due to physical causes, occurs.

TABLE.

1.	20	gms.	liver powder (Pig)	+ 60	c.c.	10 $^0/_0$	glucose	gave	in first 6 hrs.	= 0·0016	gm. CO_2.
2.	20	,,	,, ,,	+ 80	,,	,,	,,	,,	in first 6½ ,,	= 0·0044	,,
									in next 3½ ,,	= 0·0702	,,
3.	20	,,	ox liver powder	+ 30	,,	,,	,,	,,	in first 6½ ,,	= 0·0052	,,
									in next 3¾ ,,	= 0·0642	,,
4.	20	,,	pig liver powder	+ 60	,,	,,	,,	,,	in first 7½ ,,	= 0·0144	,,
5.	20	,,	ox liver powder	+ 80	,,	,,	,,	,,	in first 5½ ,,	= 0·0024	,,
									in next 5 ,,	= 0·0800	,,
6.	10	,,	pancreas	+ 50	,,	5 $^0/_0$	,,	,,	in 6 ,,	= 0·0039	,,
7.	15	,,	ox heart powder	+ 50	,,	10 $^0/_0$	,,	,,	in 6¾ ,,	= 0·0044	,,

These results show that it is immaterial whether CO_2 or H is used in procuring anærobic conditions in the experiments.

The latest paper in favour of the presence of a glycolytic enzyme in the tissues is that of Ransom(12). This observer found that frog and fowl plasma could ferment glucose under suitable conditions with the formation of alcohol and CO_2. The amount of material used in each experiment was very small, so that qualitative tests were chiefly carried out. The gas evolved was naturally assumed to be CO_2, while evidence of the presence of small amounts of alcohol was found. On repeating these experiments we succeeded in obtaining all the phenomena exactly as described. The gas obtained, however, was not pure CO_2, but mostly hydrogen. Alcohol was also found, but, unfortunately, all the samples of so-called pure glucose at our disposal gave a fairly marked reaction for alcohol[1]. In all cases bacteria were found in abundance, though often they were present in clumps, and very difficult to find.

These points suggest that the possibility of bacteria having been the cause of the results obtained by Ransom has not been excluded.

Observations on the presence of alcohol in the tissues. Sufficient has been said to explain why so many observers have maintained that the tissues contain a glycolytic enzyme capable of forming CO_2 and other products from sugar.

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[1] This probably means that the "purest" specimens of glucose are crystallised from alcohol, and that a trace remains.

The next point for consideration is: what evidence is there that traces of alcohol are found in the blood and tissues of animals? If alcohol is really found, how can its presence be explained?

As long ago as 1858 Ford(22) made the observation that traces of alcohol occur in the blood and tissues of certain animals. In a later paper(23) he supported this statement, and showed beyond any reasonable doubt that the reducing substance obtained was really alcohol. Traces of alcohol were also found in fresh milk and tissues by A. and J. Béchamp(24), and similar results were arrived at by Röhmann(25) and by Rajewsky(26). The subject has been carefully considered by Landsberg(27), who came to the conclusion that traces of alcohol do undoubtedly exist in fresh tissues, but that the amount is always small; no increase of alcohol could be detected after incubating the tissue for several days in presence of sufficient antiseptic. This observer inclined to the view that the traces of alcohol present originated in the intestinal tract, as the result of bacterial action. Kobert(28) demonstrated the presence of a reducing body in the fresh liver of the tortoise, and in many invertebrate tissues; after incubation with sugar for some time this substance increased in amount. Reach(29) claims to have found alcohol present in tissues in the form of an ester.

In the course of our experiments it was found that traces of a volatile reducing body were present in fresh tissues; this substance was found after distilling the tissue extract with picric acid and subsequently with alkali, and was probably alcohol.

A careful consideration of the literature shows that in many cases it was by no means proved that the reducing body present was alcohol. On the other hand, the experiments among others of Ford and of Landsberg leave no doubt that traces of alcohol are actually present in the fresh tissues and organs. *Where do the traces of alcohol found in the tissues originate?* We have already pointed out that certain bacteria form considerable amounts of alcohol from carbohydrates. These bacteria are present in abundance in the intestinal tract, and act on the carbohydrates of the food with the formation of alcohol and other products. It might be thought that absorption of carbohydrates would prevent this action, but there is abundant evidence that such is not the case. Macfadyen, Nencki and Sieber(30) found alcohol present in the small intestine when no alcohol was given by the mouth; they also showed that considerable amounts of dextrose were unabsorbed after the food had traversed the whole length of the small intestine.

This proves that the conditions present provide ample opportunity for bacterial action.

Since therefore alcohol is formed in the intestine, it must be absorbed and pass into the blood; this would easily account for the traces found in the tissues. There is no need to assume that alcohol is formed in the body-cells after the absorption of dextrose, since we have proof that traces of alcohol must be present in the blood and tissues. The amount of alcohol actually formed in the intestine has not been investigated, but it is possible that considerable variations, depending on the rate of absorption of sugar and other conditions, may occur. Whether this alcohol ever plays a part in certain obscure pathological processes is an interesting question for the clinician. Even if we assume that alcohol is actually formed in the tissues, the fact that a certain amount of this substance is absorbed from the intestine makes it exceedingly difficult to prove that all the alcohol found has not the same origin. Up to the present no experiments on this point have been carried out, so that the theory of alcoholic fermentation of sugar in the animal body is not in the least strengthened by the fact that blood and tissues contain traces of alcohol.

CONCLUSIONS.

1. When animal tissues or the juices and powders prepared from them are treated at 37° with glucose solution in an atmosphere of carbon dioxide or hydrogen, provided that the material remains sterile, no production of carbon dioxide occurs beyond the small amount obtainable by boiling the tissues with dilute acid.

2. It is extremely difficult to carry out such an experiment either with fresh tissues or the powders prepared from them under sterile conditions, without having recourse to antiseptics. On several occasions experiments with fresh tissues have been carried through without contamination, whereas, with powders, contamination has invariably occurred.

3. In the absence of antiseptics it is found that no appreciable evolution of carbon dioxide occurs during the first five or six hours of incubation. After that period evolution of gas begins, and gradually increases. The gas as a rule is a mixture of carbon dioxide and hydrogen, and its production is accompanied by the growth of bacteria,

which can be demonstrated in the mass, and which are capable of fermenting sugar with formation of hydrogen and carbon dioxide.

4. When a sufficient amount of an antiseptic, such as toluene, is added, and the mixture thoroughly agitated, no evolution of carbon dioxide and no development of bacteria occur. When small amounts of antiseptic are used, and when the mixture is insufficiently agitated, the onset of fermentation and bacterial growth is delayed to an extent depending on the conditions of the experiment.

5. There is, therefore, at present no satisfactory experimental evidence that alcoholic fermentation occurs in animal tissues after removal from the body. On the other hand, the foregoing experiments establish a strong presumption that no such process occurs under the conditions employed.

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THE DIACETYL REACTION FOR PROTEINS. BY ARTHUR HARDEN, F.R.S. AND DOROTHY NORRIS.

(From the Biochemical Laboratory, The Lister Institute.)

In the course of an investigation into the nature of the Voges and Proskauer reaction, Harden[1] showed that the colouration was due to the presence in the culture medium of acetylmethylcarbinol. This substance, which is a product of the growth of certain bacteria on sugars, is readily oxidised to diacetyl which then combines in the presence of alkali with some part of the protein molecule, the complex thus formed imparting to the solution a pink colour and green fluorescence.

As it is of interest to ascertain which part of the protein molecule is concerned in this reaction further experiments have been carried out with this end in view, and the results of these are embodied in the present communication. For this purpose a large number of tests have been carried out with various proteins and other substances. Beginning with more complex bodies and then testing the products of hydrolysis of those which gave a positive result, it has been found possible to trace the reaction to several bodies of simple constitution.

The following description of the examination of the protamine clupein will serve as an illustration of the methods employed. Clupein itself gives the reaction in the typical manner with a well marked fluorescence, but the mixed products obtained by heating for 14 hours with 50 % H_2SO_4 only give a pink colour, unaccompanied by fluorescence.

The products of hydrolysis were separated according to the method used by Weiss[2]. Each fraction was then tested and it was found that arginine alone of the degradation products gave the reaction.

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[1] *Proc. Roy. Soc.* B, 1906, LXXVII. p. 424.

[2] *Ztsch. physiol. Chemie*, 1907, LII. p. 107.

As a confirmatory test another sample of arginine, which was kindly given by Dr Hedin, was examined and this also gave a positive result, a pink colour unaccompanied by fluorescence.

The test was carried out by mixing a dilute solution of the protein or other substance in question with a small quantity of 10 % KOH and then adding one drop of a 1 % solution of diacetyl. In the case of simple substances it is essential to avoid both excess of alkali and of diacetyl, otherwise the diacetyl and alkali react, giving a brown colouration which in some cases may completely mask the true reaction.

Each substance appears to have its own optimum conditions; for example, using a 0·25 % solution of arginine, the best results were obtained by mixing 1 c.c. of this solution with 0·5 c.c. N. KOH, making the total volume up to 5 c.c. with water and then adding 0·05 c.c. of a 1 % solution of diacetyl. Using complex proteins such as gelatin stronger alkali is necessary, though excess of diacetyl is still to be avoided.

The reaction was usually done in the cold though heating in boiling water for one minute hastens the effect.

By these means the following substances of known structure were found to give the reaction.

Arginine $NH : C(NH_2) . NH . CH_2 . CH_2 . CH_2 . CH(NH_2) . COOH$.
Agmatine $NH : C(NH_2) . NH . CH_2 . CH_2 . CH_2 . CH_2 . NH_2$.
Creatine $NH : C(NH_2) . N . (CH_3) . CH_2 . COOH$.
Dicyanamide $NH : C(NH_2) . NH . CN$.
Guanidineacetic acid $NH : C(NH_2) . NH . CH_2 . COOH$.

With the exception of dicyanamide all the above are known to contain the guanidine grouping $NH : C(NH_2) . NHR$. In the case of dicyanamide the formula may be written $NH : C(NH_2) . NH . CN$ and here again is the guanidine structure. The reaction is however dependent to some extent upon the nature of R, for methylguanidine and aminoguanidine give decisively negative results. Free guanidine and the nitrate and hydrochloride only give the very faintest tinge of colour and this cannot be detected until the solutions have stood for several hours. The reaction is somewhat more marked with the carbonate and is quite normal with guanidineacetic acid.

It should here be noticed that no fluorescence is obtained with these simple bodies and it is possible that the fluorescence produced when complicated proteins are used may simply be due to the large molecular weight of the resulting complex. In favour of this may be cited the experiment with clupein already mentioned. Before hydrolysis this protamine gives a typical green fluorescence in addition to the pink colouration; after hydrolysis with sulphuric acid, although the pink colour due to the arginine still persists, the fluorescence is entirely lost.

The delicacy of the reaction in the case of proteins is dependent on the amount of alkali, the strength of caustic potash which will give good results with the simpler substances being altogether insufficient in the case of proteins. The following table shows this clearly in the case of Witte's peptone:

0·5 % solution of Witte's peptone	N . KOH	H_2O	1 % diacetyl	Strength of peptone in total volume	Result
4·5 c.c.	0·5 c.c.	—	0·05 c.c.	1 in 220	–
2·0 ,,	0·5 ,,	2·5 c.c.	0·05 ,,	1 in 500	–
0·5 ,,	0·5 ,,	4·0 ,,	0·05 ,,	1 in 2000	–
0·1 ,,	0·5 ,,	4·6 ,,	0·05 ,,	1 in 10000	–

Similar results were obtained with gelatin when this strength of KOH was used.

2·5 N . NaOH was next tried and the following results obtained:

0·5 % solution of Witte's peptone	2·5 N . NaOH	H_2O	1 % diacetyl	Strength of peptone in total volume	Result
5·0 c.c.	5 c.c.	—	0·05 c.c.	1 in 400	+
4·0 ,,	5 ,,	1·0 c.c.	0·05 ,,	1 in 500	+
3·0 ,,	5 ,,	2·0 ,,	0·05 ,,	1 in 700	+
2·0 ,,	5 ,,	3·0 ,,	0·05 ,,	1 in 1000	+
1·8 ,,	5 ,,	3·2 ,,	0·05 ,,	1 in 1150	+
1·6 ,,	5 ,,	3·4 ,,	0·05 ,,	1 in 1300	+
1·5 ,,	5 ,,	3·5 ,,	0·05 ,,	1 in 1400	+
1·4 ,,	5 ,,	3·6 ,,	0·05 ,,	1 in 1500	–
1·2 ,,	5 ,,	3·8 ,,	0·05 ,,	1 in 1700	–
1·0 ,,	5 ,,	4·0 ,,	0·05 ,,	1 in 2000	–

The positive results in the above table indicate a pink colour together with a green fluorescence. This last series was repeated, using 0·3 c.c. of 1 % diacetyl instead of 0·05 c.c. The positive reaction ceased at the same point of dilution as before but was somewhat more intense in colour and fluorescence.

It will be seen that a typical reaction is obtained up to 1 part in 1400 of Witte's peptone under the above conditions and that after this the results are negative.

Some experiments were then made with 60 % KOH and here there is a striking difference in the results obtained. A pink colour can be detected with 1 part in 20,000 of Witte's peptone but there is no fluorescence, and this holds even in a solution of 1 part in 1000. Taking gelatin instead of Witte's peptone a pink colour can be detected with only 1 part in 30,000. The following tables summarise these results :

0·5 % Witte's peptone	60 % KOH	H_2O	1 % diacetyl	Strength of peptone in total volume	Result
0·2 c.c.	3·5 c.c.	1·3 c.c.	0·05 c.c.	1 in 5000	+ no fluorescence.
0·1 ,,	3·5 ,,	1·4 ,,	0·05 ,,	1 in 10000	+ ,,
0·075 ,,	3·5 ,,	1·4 ,,	0·05 ,,	1 in 15000	+ ,,
0·05 ,,	3·5 ,,	1·4 ,,	0·05 ,,	1 in 20000	+ ,,

0·5 % gelatin	60 % KOH	H_2O	1 % diacetyl	Strength of gelatine in total volume	Result
0·1 c.c.	3·5 c.c.	1·4 c.c.	0·05 c.c.	1 in 10000	+ no fluorescence.
0·075 ,,	3·5 ,,	1·42 ,,	0·05 ,,	1 in 15000	+ ,,
0·05 ,,	3·5 ,,	1·45 ,,	0·05 ,,	1 in 20000	+ ,,
0·03 ,,	3·5 ,,	1·5 ,,	0·05 ,,	1 in 30000	+ ,,

It appears probable that in these very dilute solutions the protein is hydrolysed by the strong alkali used. This would account for the loss of fluorescence, the pink colour being due to the liberated arginine.

The same thing occurs in stronger solutions if time is allowed for the hydrolysis to take place. This is illustrated by the following experiments.

I. 5 c.c. of 0·5 % solution of Witte's peptone were mixed with 5 c.c. of 10 % KOH and 0·3 c.c. of 1 % diacetyl added; a typical reaction was obtained.

II. 5 c.c. of 0·5 % solution of Witte's peptone were mixed with 3·5 c.c. of 60 % KOH and this mixture was then allowed to stand over night, and then 0·3 c.c. of 1 % diacetyl added; a pink colouration only was obtained, no fluorescence.

A few experiments have been carried out using other diketones in the place of diacetyl. As yet these have yielded negative results though the γ-diketones still remain to be tried. Those actually tested were—benzil, acetylacetone, benzoylacetone, dibenzoylmethane, dibenzoylethylene. Acetone, acetaldehyde, dihydroxyacetone and glycerose also failed to give the reaction.

A list of the substances tested with diacetyl is given in the table on p. 336.

Substance	Result	Substance	Result	Substance	Result
Gelatin	+	*l*-Alanyl-*d*-alanine	–	Serine Anhydride	–
Egg Albumin	+	Di-alanyl-*l*-cystine	–	Glycine Anhydride	–
Fibrin	+	*l*-Leucinimide	–	Putrescine	–
Edestin	+	Glycyl-*l*-tyrosine	–	Cadaverine	–
Casein	+	Phenyl-alanine-*d*-alanine anhydride	–	Asparagine	–
Nuclein from Yeast	+	Glycyl-glycine	–	Urea	–
Yeast Juice	+	Biuret base	–	Uric Acid	–
Pancreatin	+	Histidine	–	Dicyanamide	+
Pepsin	+	Amine from Histidine	–	Creatine	+
Protein from Rice	+	Cystine	–	Creatinine	–
Normal Rabbit Serum	+	Amine from Cystine	–	Guanidine	?
Serum Globulin	+	Tryptophane	–	Methylguanidine	–
Globulin	+	Glycinetryptophane	–	Aminoguanidine	–
Egg Globulin	+	Amine from Tryptophane	–	Guanidine Hydrochloride	?
Clupein	+	Arginine	+	Guanidine Nitrate	?
Witte's Peptone	+	Agmatine	+	Guanidine Carbonate	?
Chapotraut's Peptone	+	Valine	–	Guanidineacetic Acid	+
Peptone Roche	–	Tyrosine	–	Semicarbazide hydrochloride	–
Peptone dry without salt	+	Aminotyrosine	–	Formamidine	–
Peptone pptd. by alc.	+	Tyrosine Anhydride	–	Succinimide	–
Peptone ex albumin	+	3·4 Dihydroxyphenylalanine	–	Succinamide	–
Peptone Riedel	+	Glycocoll	–	Acetamide	–
Peptone ex carne	+	Alanine	–	Sulphanilic Acid	–
Normal Urine	–	Phenylalanine	–	Potassium Cyanide	–
Broth	+	Leucine	–		
Meat extract	+	Glutamic Acid	–		
Spleen Autolyses	+				
Trypsin digestion of Casein	+				

SUMMARY.

The diacetyl reaction for proteins depends on the presence in the protein in question of the group $NH:C(NH_2).NH.R$.

The exact significance of R has not yet been worked out.

Only complex protein substances give any fluorescence, and this is lost if time is allowed for hydrolysis to take place before the diacetyl is added.

The authors wish to express their thanks to many colleagues for proteins and amino-acids, etc. used in the present research.

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The Fermentation of Galactose by Yeast and Yeast-juice. (Preliminary Communication.)

By ARTHUR HARDEN, F.R.S., and ROLAND V. NORRIS.

(Received July 18, 1910.)

(From the Biochemical Laboratory of the Lister Institute.)

Numerous investigators have shown that many species of yeast which ordinarily do not ferment galactose, readily acquire that property when cultivated in a medium containing that sugar.

The present communication briefly describes experiments which have been made with living yeast in this way, and with the juice obtained from this yeast. Experiments are also proceeding with yeast "killed" by acetone and other reagents (zymin), and it is hoped when these are complete to give a more detailed account of the whole investigation in a future paper.

Pure cultures of S. Carlsberg I have been used throughout these experiments. The medium employed for "training" the yeast consisted of yeast water to which was added 20 per cent. of hydrolysed lactose and 0·15 per cent. of K_2HPO_4.

Kahlbaum's galactose was used and the last traces of glucose removed by fermentation with ordinary brewers' yeast. The experiments were all carried out at 25°, those with yeast-juice being performed in the presence of toluene.

I. *Action of Untrained S. Carlsberg I on Galactose and Glucose.*

For this experiment wort cultures were employed and quantities of 1 gramme of the yeast were incubated with solutions of galactose and glucose and with water, the volume being the same in each case. The galactose was not fermented at all.

Table I.—Untrained S. Carlsberg I on Galactose and Glucose.

Experiment.	Time, in minutes.	Total fermentation. C.c. of CO_2 evolved in the time given.		
		Galactose.	Glucose.	Autofermentation.
1	65	2·5	52·9	2·4
2	35	1·2	—	1·3

II. *Fermentation of Galactose and Glucose by S. Carlsberg I grown in a Medium containing Hydrolysed Lactose.*

The results tabulated below show that the ratio of the rates of fermentation of these two sugars varies between wide limits. The conditions controlling this have not yet been ascertained.

Table II.—Comparison of Rates of Fermentation of Galactose and Glucose by S. Carlsberg I after Cultivation in a Medium containing Hydrolysed Lactose.

Experiment.	Mean rate of fermentation for 30 minutes. C.c. of CO_2 evolved in 5 minutes.		Ratio $\frac{\text{Glucose}}{\text{Galactose}}$.
	Galactose.	Glucose.	
3	2·81	2·35	0·84
4	10·21	19·25	1·86
5	1·52	2·32	1·52
6	2·52	3·52	1·39
7	4·00	6·63	1·65
8	1·65	2·18	1·31
9	1·37	3·50	2·55
10	2·70	7·60	2·83
11	5·35	15·00	2·79

III. *Fermentation of Galactose by Yeast-juice.*

The juice was obtained from S. Carlsberg I cultivated in the medium described above. The yeast was well washed with water and then ground in the usual manner.

Experiment 12.—Two quantities of 20 c.c. juice, to one of which 1 gramme of galactose had been added, were incubated at 25° in presence of toluene.

Experiment 13.—Three quantities of 25 c.c. of a different juice were incubated as above; to one was added 2 grammes galactose, to the second 2 grammes glucose, and to the third 1 c.c. H_2O.

Table III.—Fermentation of Galactose by Yeast Juice obtained from Trained S. Carlsberg I.

Experiment.	Fermentation in 1 hour. C.c. of CO_2 evolved.		
	Galactose.	Autofermentation.	Glucose.
12	49·0	8·4	—
13	39·3	8·6	25·7

These experiments clearly show that galactose is readily fermented by the juice obtained from yeast which has acquired the property of fermenting this sugar.

IV. *Total Fermentation of Galactose and Glucose by Yeast-juice.*

Experiment 14.—Two quantities of 25 c.c. of the same yeast-juice as used in Experiment 13 were incubated with 2 grammes galactose and 2 grammes glucose respectively in presence of toluene.

The initial rate of the galactose solution was higher than that of the glucose, but the total volume of gas evolved was slightly greater in the case of glucose. Fermentation had ceased in both cases after 19 hours.

Table IV.—Total Fermentation of Galactose and Glucose by Yeast-juice from Trained S. Carlsberg I.

Initial rate. C.c. in 5 minutes.		Total fermentation.		Ratio of totals.	
Galactose.	Glucose.	Galactose.	Glucose.	Galactose.	Glucose.
3·7	3·1	94·9	99·2	0·95	1·00

V. *Fermentation of Galactose by Yeast-juice in presence of Phosphate. Relation of extra Carbon Dioxide evolved to Phosphate added.*

The addition of phosphate to a fermenting mixture of galactose and yeast-juice produces an acceleration in the rate, and an extra amount of carbon dioxide is evolved which is equivalent to the phosphate added. As in the case of glucose, the phosphate is converted into an organic compound which cannot be precipitated with magnesium citrate mixture. The compound formed can be isolated in the form of its lead salt as described by Young for glucose.[*]

The extra carbon dioxide was evolved much more rapidly in the case of glucose than with galactose with the particular sample of juice employed.

Experiment 15.—Two quantities of 25 c.c. yeast-juice were incubated with 1 gramme of the sugar under investigation until a constant rate was obtained and 2·5 c.c. of a 0·3 molecular solution of sodium phosphate then added to each.

Table V (*a*).—Acceleration of Rate of Fermentation of Galactose and Glucose by Yeast-juice on addition of Phosphate.

Experiment 15.

Time after addition of phosphate, in minutes.	CO_2 evolved in preceding 5 minutes.	
	Galactose.	Glucose.
(Rate previous to addition)	0·8	1·3
5	5·3	8·3
10	3·6	7·9
15	3·4	5·7
20	2·8	2·6
25	2·4	1·9
30	2·5	1·3
35	2·1	1·3
40	2·0	
45	1·8	
50	1·5	
55	0·9	

Table V (*b*).—Equivalence of extra CO_2 evolved to Phosphate added in above Experiment.

	Galactose.	Glucose.
Maximum rate obtained	5·3	8·3
Final rate	0·9	1·3
Total gas measured	29·1 in 1 hour	27·7 in 30 mins.
Correction for rate without phosphate	10·8	7·8
CO_2 due to phosphate	18·3	19·9
„ at N.T.P.	16·7	18·2

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[*] 'Roy. Soc. Proc.,' B, 1909, vol. 81, p. 528.

The phosphate added was equivalent to 16·7 c.c. of CO_2 at N.T.P. in the ratio $Na_2HPO_4 : CO_2$.

The phenomena observed with galactose are therefore precisely the same as those exhibited by glucose.

VI. *Formation of an Organic Phosphate.*

25 c.c. of juice + 2 grammes galactose were incubated and phosphate added in small quantities until no further acceleration could be produced.

When the rate had fallen to its original value, the free phosphate in the boiled and filtered liquid was estimated.

Free phosphate found was equivalent to	0·1400	gramme $Mg_2P_2O_7$.
The phosphate added corresponded to	0·3953	„ „

Hence phosphate equivalent to 0·2553 gramme $Mg_2P_2O_7$ has been converted into a form not precipitable by magnesium citrate mixture. The constitution and properties of this compound are under investigation.

VII. *Fermentation of Galactose by Yeast-juice in Presence of Arsenate.*

As with glucose the addition of small quantities of sodium arsenate to a fermenting mixture of galactose and yeast-juice produces an acceleration, but hitherto it has only been found possible to double the rate in this way.

Conclusions.

1. The statement by various workers that some yeasts can be trained to ferment galactose by cultivation in a medium containing that sugar, has been confirmed.
2. Such a trained yeast yields a juice capable of fermenting galactose.
3. A fermenting mixture of yeast-juice and galactose reacts with phosphate in a similar manner to yeast-juice and glucose. The rate is accelerated, an extra amount of CO_2, equivalent to the phosphate added, is evolved and the rate then again becomes normal. The phosphate is converted into an organic compound not precipitable by magnesium citrate mixture.
4. The fermentation of galactose by yeast-juice is also accelerated by the addition of small quantities of sodium arsenate.

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110. "The influence of salts on the autofermentation of yeast." (Preliminary note.) By Arthur Harden and Sydney G. Paine.

It has been found by one of the authors, during an investigation of the permeability of the yeast-cell envelope, that the entrance of such salts as sodium chloride and sodium phosphate is imperceptible when dilute solutions are allowed to act for a short time, and comparatively small even after prolonged immersion of the cells. When, however, more concentrated solutions are employed, an appreciable entrance of the salts is indicated.

These facts, in view of the recent work of H. E. and E. F. Armstrong (*Proc. Roy. Soc.*, 1910, *B*, **82**, 588), therefore suggested the advisability of ascertaining the effect of such salts on the autofermentation of the yeast.

For the experiments here given, top yeast obtained from a London brewery was freed from wort by the simple expedient of pressing, which has been found to give practically quantitative removal of the interstitial liquid; washing was avoided in order that osmotic equilibrium of the cell contents might remain undisturbed.

Three to five grams of such yeast are accurately weighed out for each experiment, wrapped in tissue paper, and dropped into a flask containing a measured quantity of the liquid under investigation, which has previously been saturated with carbon dioxide at 25°. The gas evolved by autofermentation of the yeast is measured periodically by means of the apparatus described by Harden, Thompson, and Young (*Bio-Chem. J.*, 1910, **5**, 230).

In experiments with sodium chloride and ammonium sulphate it has been found that when these salts are present in decimolar concentration no effect is produced on the autofermentation, but when molar solutions are employed the rate of fermentation is very considerably enhanced during the first four or five hours, so that the volume of carbon dioxide yielded in presence of the salt is three times as great as that given by a water control. At this point, however, the autofermentation in the former case comes abruptly to an end, and eventually after four or five days the volume of gas given in the two cases is approximately the same.

A similar result has also been obtained with a number of other salts, including phosphates, arsenates, acetates, and citrates.

In a recent paper by C. Neuberg and A. Hildesheimer (*Biochem. Zeitsch.*, 1911, **31**, 170) it is stated that salts of pyruvic acid are fermentable by yeast, and that this property is also exhibited by salts of glyceric and lactic acids. Similar experiments have been carried out with these salts, and these have shown that the two latter give precisely the same phenomenon as was found in the case of sodium chloride, whilst with potassium pyruvate the rate of fermentation during the first four hours is exactly comparable with that in a sodium chloride control. At this point, however, the fermentation in presence of the latter salt slackens, while the evolution of gas from the pyruvate solution continues and only gradually diminishes. Eventually after four days some 60 c.c. of carbon dioxide are yielded in presence of 2·4 grams of potassium pyruvate in excess of the volume given by the control.

The provisional conclusion of Neuberg and Hildesheimer that these salts undergo fermentation by yeast cannot, therefore, be accepted until experimental proof is forthcoming that the carbon dioxide is actually derived from them, and not from the constituents of the yeast.

The increase in the rate of autofermentation produced by the addition of salts to living yeast may be explicable on the lines suggested by H. E. and E. F. Armstrong, or it may be that, as a result of plasmolysis of the cells, the glycogen is thrown into more intimate contact with the glycogenase.

The acceleration does not appear to be due to a stimulating influence on the glycogenase, since when added to yeast juice in equivalent concentration sodium chloride tends to inhibit, rather than to enhance, the rate of autofermentation.

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APPARATUS FOR COLLECTING AND MEASURING THE GASES EVOLVED DURING FERMENTATION

BY

A. HARDEN, J. THOMPSON AND W. J. YOUNG

APPARATUS FOR COLLECTING AND MEASURING THE GASES EVOLVED DURING FERMENTATION

By A. HARDEN, J. THOMPSON and W. J. YOUNG.

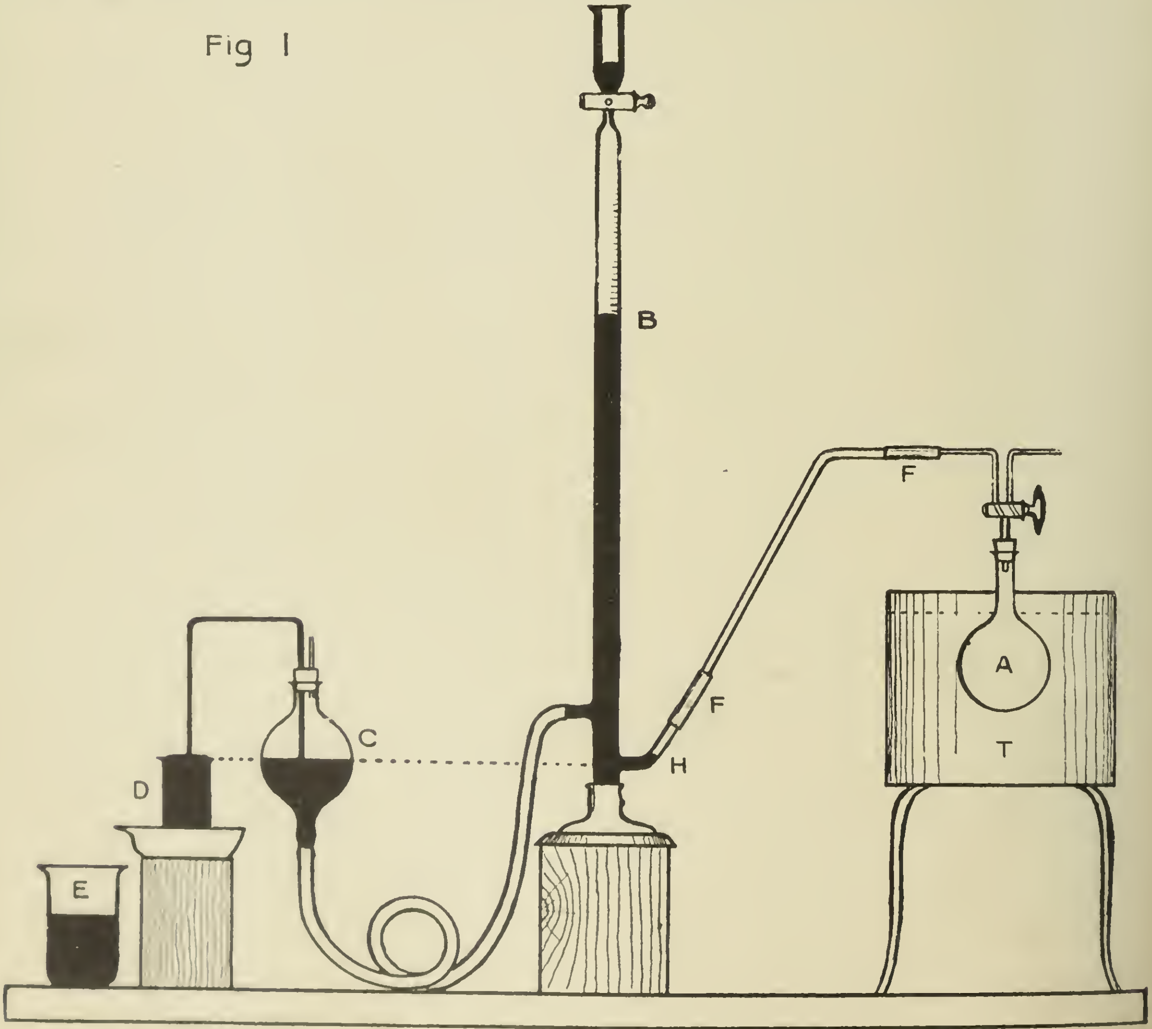
From the Bio-Chemical Laboratory, Lister Institute

(Received August 2nd, 1910)

In measuring the total quantity and rate of evolution of gases evolved during fermentation, it is essential to keep the pressure in the fermentation flask as nearly constant as possible throughout the experiment. Two forms of apparatus have been in use in this laboratory for some time for this purpose, and have been found to be very simple and convenient.

1. The first form is intended for the measurement of comparatively small volumes of gas, such as those evolved in the fermentation of sugars by yeast juice,[1] and is illustrated in the accompanying figure 1.

Fig I



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[1] Harden and Young. *Roy. Soc. Proc. B.*, 1906, 77, 406.

The fermentation flask (A) of 100-200 c.c. capacity, placed in the thermostat (T), is fitted with a three-way tap connected by glass tube and rubber with a Schiff's azotometer of 100 c.c. capacity, graduated to 0·2 c.c. It is essential that the rubber connections (F, F) should be as short as possible, owing to the high permeability of rubber for carbon dioxide, but at the same time it is necessary to allow so much play that the flask can be removed from the bath and shaken by hand, or mechanically agitated in the bath. The reservoir (C) of the azotometer is connected by a syphon, passing through a doubly-bored stopper, with a small cylinder or beaker (D) which stands in a flat dish of glass or porcelain provided with a spout from which mercury can flow into a collecting vessel (E). The effect of this arrangement is that the mercury displaced from B passes through the syphon into D and overflows into the porcelain dish and so into E. The mercury at C and H, therefore, remains at the level of the top of D, and this is adjusted once for all, so that the mercury just extends to the bend of the tube H, and consequently the pressure in the flask is maintained at that of the atmosphere.

The volume of gas is read on B at the reduced pressure corresponding to the height of the column BH, and is corrected by means of a table. (See below.)

When the azotometer is full of gas, the tap on A is closed, the syphon removed, the reservoir filled with the mercury from E, and the gas displaced by raising the reservoir. The syphon is then replaced, and started by means of a pressure bulb attached to a short tube passing through the stopper of the reservoir. The tap on A is then re-opened, and the collection of gas resumed as before. If required, a sample of the gas can be taken for analysis by means of the mercury cup at the top of the azotometer.

The table of corrections is constructed for an atmospheric pressure of 760 mm. by measuring the height of each cubic centimetre graduation above H, subtracting this from 760, and then calculating the corrected volume corresponding with each graduation. The corrected numbers for the intermediate graduations are found by interpolation. A table of this kind is sufficiently accurate for most purposes; when greater accuracy is required the readings must be specially corrected for the prevailing atmospheric pressure.

The rate of fermentation of a sugar by yeast-juice is determined by the aid of this apparatus in the following matter. The azotometer is filled with mercury and the syphon adjusted in C. The desired mixture of yeast-juice, sugar and toluene is placed in A, brought to the temperature of the thermostat and saturated with carbon dioxide by removing the flask, filling it with carbon dioxide from a Kipp, replacing the stopper carrying the tap and shaking, and repeating this process until saturation is effected. If desired, the flask can be fitted with inlet and outlet tubes and the carbon dioxide blown through without removing the flask from the bath, but this is not so convenient in the case of yeast-juice as the method described above.

Since yeast-juice readily becomes supersaturated with carbon dioxide, the flask is vigorously shaken for about half a minute before each reading. The flask is then replaced in the thermostat, allowed to stand for one minute to re-establish temperature equilibrium, and the volume of gas in the azotometer read. At the expiration of the desired interval from the time of first shaking, the process is exactly repeated and the volume of gas evolved during the interval thus ascertained.

So strong is the tendency to supersaturation in the case of yeast-juice that scarcely any gas is evolved during 5-10 minutes, the whole volume produced during this time being retained in the liquid and only evolved when this is vigorously agitated.

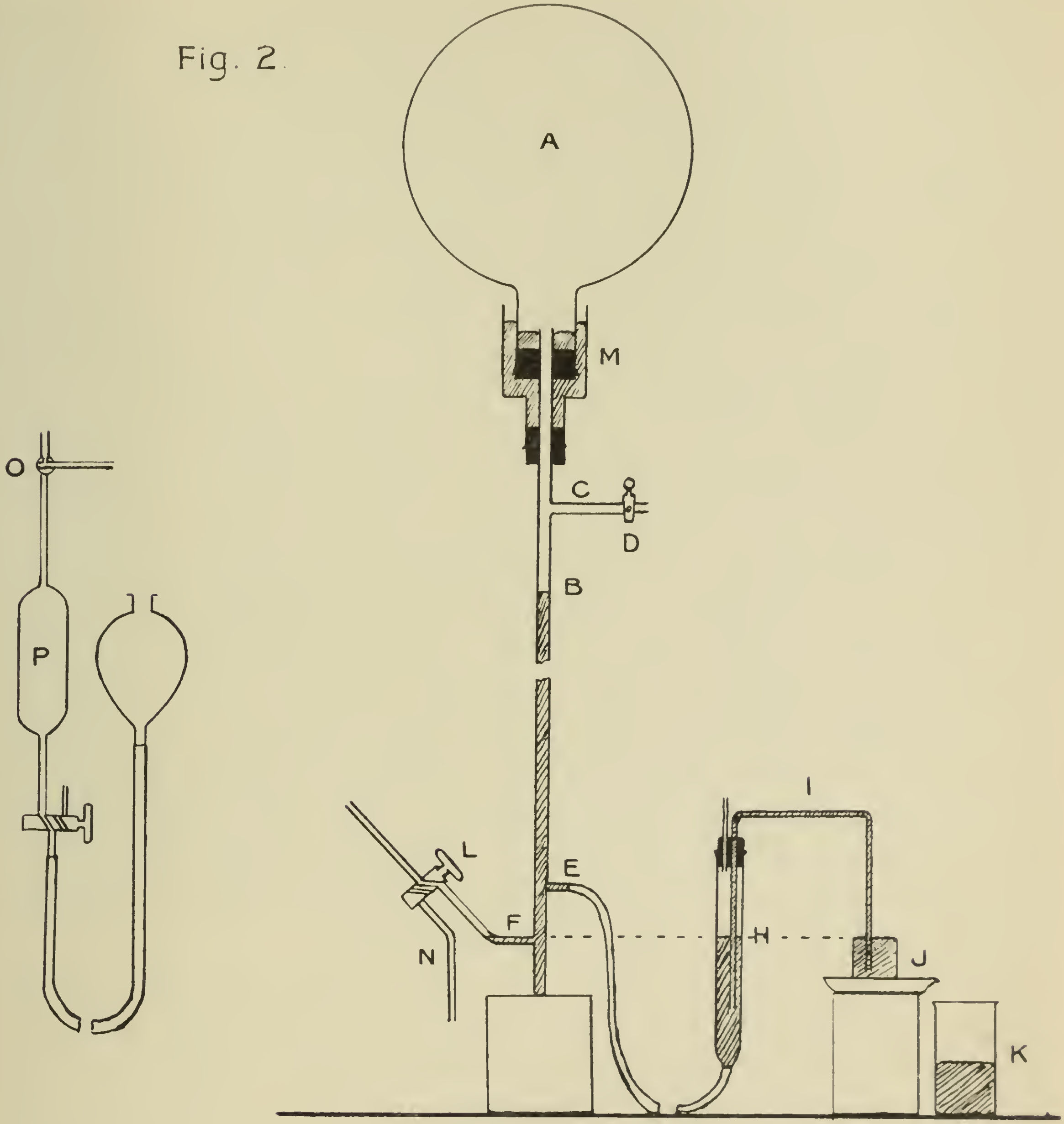
This method works extremely well so long as the volume of gas evolved does not exceed about 30 c.c. per 5 minutes, and does not fall below about 0·5 c.c.

2. The second form of apparatus is intended for the collection and measurement of the comparatively large volumes of gas which are evolved by the action of bacteria on quantities of about 20 grams of sugar, the object being the collection and measurement over mercury of a large volume of gas (3-6 litres) without employing an unmanageable amount of mercury. The apparatus is shown in fig. 2.

A large inverted bolthead (A) (3-7 litres in capacity) is fitted with a rubber stopper carrying a vertical tube (B) about 1 metre in length and 10 mm. in external diameter. This tube projects about 2 cm. inside the stopper of the flask, and carries near the top a side piece (C) fitted with a good mercury sealed glass tap (D). B is sealed at the lower end, near which are two side tubes arranged as in Schiff's azotometer (E, F). At E is attached a rubber tube and a cylindrical mercury reservoir (H) about

20 mm. in diameter and 180-200 mm. in length, and this is fitted with a syphon and overflow cylinder (I, J K) exactly like those employed for apparatus 1. The side tube (F) carries a three-way tap (L).

Fig. 2.



In order to prevent leakage, the rubber stopper of the flask A is immersed in mercury contained in a cup (M) carried on the vertical tube B by means of a rubber stopper. In order to prepare the apparatus for use, mercury is poured into the reservoir (H), and the lower part of the tube B and side tube F up to the tap are filled with mercury, which is also allowed to flow out of the upper end of B and fill the space immediately above the stopper of the bolthead. This prevents the gas from coming into contact with rubber after it has once passed into the apparatus. The side tube C is then connected with a Fleuss pump or a mercury pump and the whole apparatus exhausted. Mercury then rises in B to the barometer height above that in H. As soon as the exhaustion is as complete as possible, D is closed; H is fitted with its syphon, and J is adjusted so that the level of the mercury is at the same height as the point at which F enters B. The height of the column BF subtracted from the height of the barometer gives the residual pressure, if any, in the apparatus, and is measured by a vertical millimetre scale. The fermentation flask, placed in an appropriate incubator or thermostat, is connected by rubber to the tube carrying the tap L, and the air of the space above the liquid in it is swept out, if this is desired, by a suitable gas through the limb N. The tap L is then turned so as to connect the flask with B, and the collection of the gas then proceeds regularly until the experiment is interrupted. Any mercury carried up by the bubbles of gas strikes against the concave surface of the bolthead and falls back again into the tube. The diameter of the side tube F must be decidedly less than that of B, or the bubbles of gas entering B drive too much mercury before them and cause violent oscillation of the column. At the close of the experiment the pressure and temperature of the gas are read off and its corrected dry volume ascertained by calculation from the known capacity of the apparatus. The calibration of the large flask is effected by means of water, and that of the tube B by means of mercury. It is necessary to know the volume of the bolthead as arranged for the experiment, and of the side tube C and tube B down to a fixed point, and the capacity per centimetre below this. The actual internal volume of the apparatus when the mercury is standing at any height above H is then easily ascertained.

A sample of gas for analysis is taken at D by means of the pipette P, of about 100 c.c. capacity. This is attached at D, and the air from the intervening tube removed by using the pipette and its reservoir as a mercury pump, the gas removed being swept out of the three-way tap O. After this process has been completed, the reservoir of the pipette is lowered and the taps D and O are opened; gas flows from A to P, and as soon as no more gas passes over, the taps are closed and the pipette disconnected. The sample can then readily be brought into a gas analysis apparatus. The total error in the measurement of the volume need not exceed 0·5 per cent.

The space above the liquid in the fermentation flask is kept as small as possible, and should not be more than 100-200 c.c. when 1 litre of liquid is employed. The composition of the gas remaining in this space can be ascertained with sufficient accuracy by rapidly removing a sample with the pipette from N; the slight error due to evolution of carbon dioxide or other gas from the liquid in the flask during this process being as a rule negligible. Finally, in calculating the volume of gas actually evolved during the experiment, it is necessary to allow for the volume of gas originally present in the flask; for the gas dissolved in the liquid in the flask; and for the gas corresponding with any residual pressure in the apparatus at the commencement of the fermentation.

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No 21

Alcoholic Fermentation.

(Experimental Demonstration.)

By ARTHUR HARDEN and W. J. YOUNG.

The following conclusions have been drawn by the authors from their work upon this subject:—

1. *Enzyme and co-enzyme.*—At least two agents are concerned in the alcoholic fermentation of glucose by Buchner's yeast-juice; (*a*) the *enzyme*, which is thermolabile and non-dialysable; (*b*) the *co-enzyme*, which is thermostable and dialysable.

During fermentation both the enzyme and the co-enzyme gradually disappear, and the cessation of fermentation may be due to the complete disappearance of either or both.

2. *Function of phosphates in fermentation.*—(*A*) The fermentation of glucose by yeast-juice proceeds according to the following equations, phosphate being used up according to (i), and regenerated according to (ii.).

$$\text{(i)}\ 2C_6H_{12}O_6+2PO_4HR^1_2=2CO_2+2C_2H_6O+C_6H_{10}O_4(PO_4R^1_2)_2+2H_2O$$

$$\text{(ii)}\ C_6H_{10}O_4(PO_4R^1_2)_2+2H_2O_2=C_6H_{12}O_6+2PO_4HR^1_2.$$

These equations are deduced from the following phenomena:-

(*a*) When a soluble phosphate is added to a mixture of yeast-juice and glucose a period of accelerated activity is observed, during which the extra amounts of carbon dioxide and alcohol formed are equivalent to the phosphate added, as expressed in equation (i.).

(*b*) When glucose is fermented in the presence of excess of free phosphate, exactly half of it is rapidly transformed into carbon dioxide and alcohol.

(*c*) The phosphate is converted into a compound of the formula $C_6H_{10}O_4$ $(PO_4R^1{}_2)_2$, which has been isolated in the form of its metallic salts, and is probably a hexosephosphate.

(*d*) This complex phosphate undergoes hydrolysis by the action of an enzyme contained in the juice with formation of free phosphate and a sugar.

(*e*) As long as fermentation is proceeding actively, the concentration of phosphate remains low, owing to the occurrence of reaction (i.). When fermentation slackens, free phosphate accumulates, according to equation (ii.).

(*B*) According to these equations, the presence of phosphate is essential for fermentation. Complete proof of this has not yet been obtained, since the total absence of free or available phosphate has not been experimentally realised. When, however, the amount of phosphate present is very low, the total fermentation produced is also low, and is very greatly increased by the addition of a small quantity of phosphate.

(*C*) *Effect of excess of phosphate.*—Addition of phosphate beyond a certain limit causes a partial or complete inhibition of fermentation.

3. *Mannose* behaves towards yeast-juice and phosphates both qualitatively and quantitatively in a similar manner to glucose.

4. When *fructose* is substituted for glucose, reaction (i.) proceeds much more rapidly, and a much greater concentration of phosphate is required to inhibit the fermentation.

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The Alcoholic Ferment of Yeast-juice. Part VI.—*The Influence of Arsenates and Arsenites on the Fermentation of the Sugars by Yeast-juice.*

By ARTHUR HARDEN, F.R.S., and W. J. YOUNG (Biochemical Laboratory Lister Institute).

(Received January 17,—Read March 2, 1911.)

The rate of fermentation of a simple sugar by yeast-juice undergoes a temporary acceleration when a soluble phosphate is added.* The close chemical analogy which exists between phosphates and arsenates suggested the idea that this similarity might extend to the effect of arsenates on fermentation, and accordingly experiments were made to test the point. It was thus found that arsenates have a great effect on fermentation, but subsequent work has shown that it is of a totally different nature from that exerted by phosphates.

I. *Influence of Arsenate on the Rate of Fermentation.*

When a solution of sodium arsenate is added to a mixture of glucose, mannose or fructose with yeast-juice, which has attained a steady rate of fermentation, an immediate large increase in the rate of evolution of carbon dioxide is produced. The rate, as a rule, rises for a short period, attains a maximum, and then very gradually diminishes. A typical example is the following.

Experiment 1.—The experiments throughout were carried out at 25° in presence of toluene, the solutions being previously saturated with carbon dioxide. The standard solution of sodium arsenate employed was approximately 0·3 molar, and was prepared by dissolving 5·312 grammes of crystallised sodium arsenate in water, and making up to 100 c.c. Two separate mixtures were made, each containing 25 c.c. yeast-juice and 2·5 grm. of glucose, and were incubated until a steady rate of fermentation had been attained. There were then added (*a*) 25 c.c. of water and 2·5 grm. glucose; (*b*) 20 c.c. of water + 5 c.c. standard arsenate solution and 2·5 grm. glucose.

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* Harden and Young, 'Roy. Soc. Proc.,' B, 1906, vol. 77, p. 410.

The rates of evolution of carbon dioxide were then observed, the apparatus previously described being employed.

Time.	*a.*		*b.*	
	Total gas.	Average rate per 5 minutes during interval.	Total gas.	Average rate per 5 minutes during interval.
5	2·1	2·1	23·3	23·3
10	4·9	2·8	47·8	24·5
15	8	3·1	72·5	24·7
20	10·7	2·7	99·5	27
30	15·9	2·6	154·1	27·3
40	21·4	2·8	208·4	27·15
50	26·5	2·55	259·3	25·45
65	34·5	2·7	332·2	24·3
95	50·6	2·7	459·0	21·1

A striking feature of the effect of the addition of a phosphate to yeast-juice is that the marked acceleration only continues until an amount of carbon dioxide has been evolved which is chemically equivalent to the phosphate added. Moreover, at the close of this period of enhanced fermentation, the added phosphate is no longer present in a form precipitable by magnesium citrate mixture, but has become converted into a hexose-phosphate. Neither of these phenomena occurs when an arsenate is substituted for the phosphate. The enhanced rate of fermentation continues long after an equivalent of carbon dioxide has been evolved, and no organic combination of arsenic is formed.

The first of these statements follows from the results of Experiment 1. Here the arsenate added was equivalent to 36 c.c. of carbon dioxide in the ratio $Na_2HAsO_4 : CO_2$, whilst the extra carbon dioxide evolved in the presence of the arsenate amounted to 408·4 c.c. in 95 minutes, and the rate at the end of that time was still more than seven times that of the juice without arsenate.

The sharp contrast between the actions of arsenate and phosphate is clearly shown when the effects of equivalent amounts of phosphate and arsenate on the same sample of yeast-juice are directly compared, as is done in the following experiment.

Experiment 2.—(1) 20 c.c. yeast-juice + 5 c.c. of 0·3 molar phosphate; (2) 20 c.c. yeast-juice + 5 c.c. of 0·3 molar arsenate; (3) 20 c.c. yeast-juice + 0·75 c.c. of 0·3 molar arsenate.

Excess of sugar was present in each case, and the solutions of phosphate and arsenate were each equivalent to 36 c.c. of carbon dioxide for 5 c.c. of the solution. The normal rate of fermentation of the juice was 1·8 c.c. per five minutes.

Time.	1.		2.		3.	
	Total.	Rate per 5 minutes.	Total.	Rate per 5 minutes.	Total.	Rate per 5 minutes.
5	7·1	7·1	11·4	11·4	21·7	21·7
10	19·8	12·7	26·5	15·1	46	24·3
15	36·1	16·3	43	16·5	71	25
20	43·8	7·7	59	16	95·3	24·3
25	45·7	1·9	75	16·3	119·8	24·5
30	47·5	1·8				

Calculating the extra amount of carbon dioxide evolved in each case beyond that due to the normal rate of the juice, it is found that, in presence of phosphate, this amounts to 36·7 c.c., and that the rate has fallen to that of the original juice. In presence of arsenate, although the extra amount of carbon dioxide is 66·3 c.c., the rate has remained unaltered at the high value of 16. The disparity between this extra amount of carbon dioxide and the equivalent of the arsenate added is still more strikingly illustrated when only a small amount of arsenate is added. This is shown in No. (3) above, to which only 0·75 c.c. of arsenate, equivalent to 5·4 c.c. of carbon dioxide, was added. In spite of this the extra amount of carbon dioxide evolved in 25 minutes was 120·8 c.c., and the rate at the end of that time was still 24·5 c.c. per 5 minutes.

In order to show that the whole of the arsenate is still present in its original state after the evolution of an equivalent amount of carbon dioxide, the following experiment was made:—

Experiment 3.—Four quantities of 25 c.c. of yeast-juice + 2·5 grm. of glucose were incubated until a steady rate had been attained.

To these were added:—

(1) 5 c.c. water.

(2) 5 c.c. of sodium arsenate solution, yielding 0·2211 grm. of $Mg_2As_2O_7$, and equivalent to 35 c.c. of carbon dioxide.

(3) 5 c.c. of sodium arsenate, as above, and 2·5 grm. of glucose.

(4) 5 c.c. of sodium phosphate equivalent to the arsenate added to (2), and yielding 0·1553 grm. of $Mg_2P_2O_7$.

The fermentations of 1 and 2 were then observed until the evolution of carbon dioxide from (2) was approximately equal to that from (1) + 35 c.c., and the two liquids were then boiled and filtered and the weight of precipitate yielded by magnesium citrate mixture ascertained.

Liquid (3) was allowed to ferment until a total of 713 c.c. had been evolved, and was then boiled and treated as above.

Finally (4), which was intended to demonstrate the different behaviour of phosphate, was allowed to ferment until the rate again became normal, and was then boiled and precipitated with magnesium citrate mixture.

The following table shows the total weight of precipitate produced in each case by magnesium citrate mixture:—

	Original juice.	Added.	Total before incubation.	Total after incubation.	Difference.
1	0·0678	0	0·0678	0·0678	0
2	0·0678	0·2211	0·2889	0·3007	+0·0118
3	0·0678	0·2211	0·2889	0·3513	+0·0624
4	0·0678	0·1553	0·2231	0·1025	−0·1206

These numbers clearly show that the whole of the arsenate remains precipitable by magnesium citrate mixture, even after prolonged fermentation, whereas the phosphate is converted into a non-precipitable form.

This fact is, however, not sufficient to exclude the possibility of the formation of an arsenic analogue of a hexosephosphate, since this might be precipitable by magnesium citrate mixture.

Experiment 4.—The precipitate obtained from another experiment conducted on similar lines to (2) was therefore carefully washed, dried at 100°, mixed with copper oxide, and submitted to combustion in a current of oxygen; 0·3621 grm. gave 0·0069 grm. of CO_2, corresponding with 0·052 per cent. of carbon. A magnesium hexose-arsenate would contain 15·3 per cent. of carbon, so that the result of this analysis shows that no appreciable amount of such a compound is present. The trace of carbon actually found was no doubt derived from occluded matter from the boiled yeast-juice in which the precipitate was produced. The arsenate therefore remains free throughout the fermentation.

2. *The Products of Fermentation in Presence of Arsenate.*

In order to ascertain whether the reaction which occurred in the presence of arsenate was the normal decomposition of sugar into alcohol and carbon dioxide, the ratio of these products was determined for an arsenate fermentation.

Experiment 5.—75 c.c. of yeast-juice were incubated with 8 grm. of glucose and 15 c.c. of 0·1 molar sodium bicarbonate solution in order to remove all free acid and free phosphate. In one portion of this the alcohol was estimated at once by distillation. To each of two other equal portions, 1 c.c. of arsenate solution was added, and both were incubated at 25° for 1·5 hours. During this period 185·3 c.c. of pure carbon dioxide, measured moist at 20·8° and 763·6 mm., were evolved, weighing 0·3303 grm. At the end of this time the total alcohol was estimated in one of the portions, and the alcohol produced in presence of arsenate thus ascertained by subtracting that originally present. The following were the results:—

	Before incubation.	After incubation.	Difference.	Ratio.
Alcohol	0·4898	0·7924	0·3026	0·92
Carbon dioxide	0	0·3303	0·3303	

The theoretical ratio is 1·04. The value obtained agrees well with the ratio previously found by similar methods for the normal fermentation of glucose by yeast-juice.

3. *Effect of the Concentration of Arsenate on the Maximum Rate of Fermentation Attainable.*

The rate attained increases with the concentration of arsenate until a certain limit is reached. A further increase beyond this point diminishes the rate of fermentation, so that just as in the case of phosphate there is a certain optimum concentration of arsenate, corresponding with which there is a maximum rate of fermentation.

In carrying out these estimations of rate of fermentation, a mixture of glucose with yeast-juice, 10 grm. to 100 c.c., was incubated until the rate of evolution of carbon dioxide had become constant, and all free phosphate had been converted into hexosephosphate. The standard sodium arsenate solution (0·3 molar), containing 10 grm. of glucose per 100 c.c. and saturated with carbon dioxide at 25°, was then added in the desired quantity. Since sodium arsenate solution after saturation with carbon dioxide contains an equilibrium mixture of the two arsenates and sodium bicarbonate, the amount of the latter present in the arsenate solution used was previously estimated by neutralising a known quantity with hydrochloric acid and measuring the carbon dioxide evolved. A solution of sodium bicarbonate, also containing 10 grm. of glucose per 100 c.c., was then made up which yielded the same amount of carbon dioxide when neutralised as the arsenate solution. The liquid in each flask was made up to the same volume with this sodium bicarbonate solution. The fermentation flasks containing the proper quantities of arsenate solution and bicarbonate were then incubated at 25° and the rates of fermentation observed. The effect of bicarbonate is to cause a slight increase in the rate of fermentation, owing to a diminution of the acidity of the juice (Buchner).

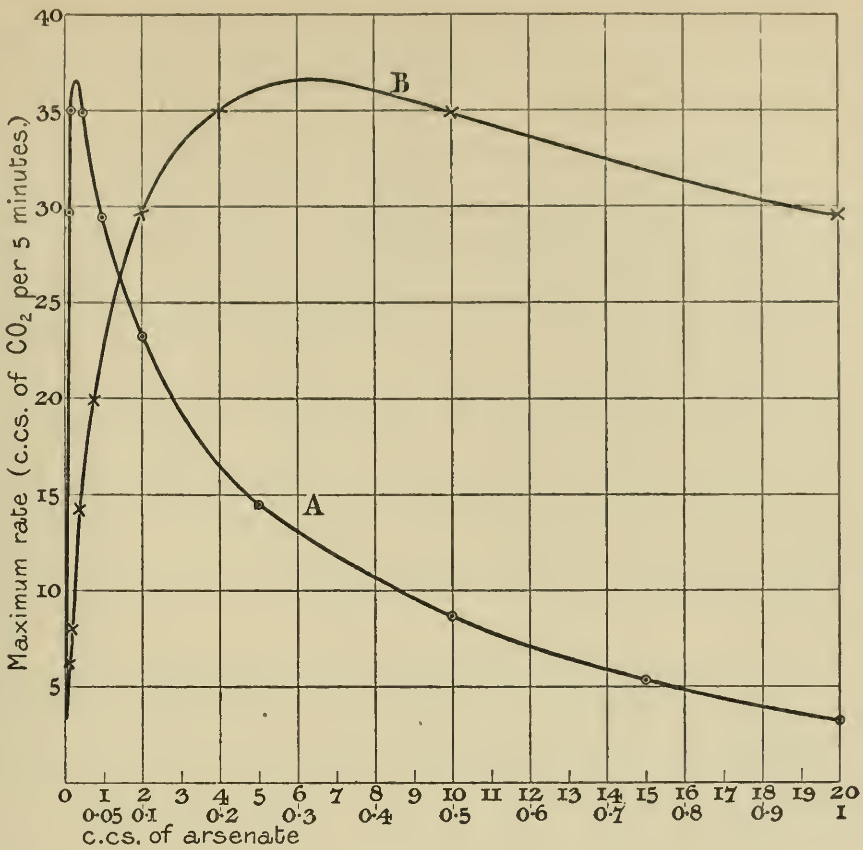
The following results, all obtained from a single sample of yeast-juice, are typical, and show the nature of the relation between concentration of arsenate and rate of fermentation.

When the concentration of arsenate is very low, the rate falls off very rapidly from the maximum, and the numbers obtained in these cases can only be regarded as approximate. This is the case in the present set of observations for amounts of arsenate less than 0·1 c.c.

Experiment 6.—20 c.c. of yeast-juice + x c.c. of 0·3 molar arsenate + $(20-x)$ c.c. sodium bicarbonate solution. Glucose, 4 grm

Arsenate solution per 40 c.c. mixture.	Molar concentration of arsenate in fermenting mixture.	Maximum rate of fermentation.
c.c.		c.c. per 5 minutes.
0	0	3·5
0·005	0·000037	6·3
0·01	0·000075	8·0
0·02	0·00015	14·2
0·04	0·0003	19·9
0·1	0·00075	29·7
0·2	0·0015	35·0
0·5	0·00375	34·9
1	0·0075	29·5
2	0·015	23·2
5	0·0375	14·5
10	0·075	8·7
15	0·1125	5·3
20	0·15	3·2

The same results are exhibited graphically in the accompanying curves, in which the rate is plotted against the cubic centimetres of arsenate present in the mixture. Curve B represents the earlier portion of A, plotted with a twenty-fold scale of abscissæ.



The striking feature of the curve is the rapid rise of rate with the concentration, followed by a fall, at first rapid and then gradual. The reason for this fall is discussed later on.

The smallest amount of arsenate recognisable by a change of rate of fermentation in this experiment corresponded with the presence of 0·11 mgrm. of arsenic in the fermenting liquid.

Both the optimum amount of arsenate and the maximum rate of fermentation produced by it vary with the sample of yeast-juice employed, and the following numbers illustrate this point:—

Experiment.	Volume.	Juice alone.	Maximum obtained.	Ratio.	Optimum molar concentration of arsenate.
		c.c. per 5 minutes.			
7	20	3·5	36·5	10·4	0·00225
8	20	1·3	14·2	10·9	0·01125
9	20	1·6	25	15·6	0·005625

4. *Effect of the Addition of Arsenate on the Total Fermentation produced by Yeast-juice.*

When arsenate is added to yeast-juice and sugar, the rate of fermentation more or less rapidly reaches the maximum corresponding with the concentration of arsenate employed. This rate is maintained for a varying time, according to the amount added, and then slowly falls off until ultimately fermentation ceases.

When the concentration of arsenate is comparatively high, fermentation ceases much sooner than in the absence of arsenate, and this appears to be due to some direct action on the fermenting complex, similar to that which was observed by Buchner as resulting from the addition of potassium arsenite, and which is discussed later on. When, on the other hand, a small concentration of arsenate is used a high rate is produced, fermentation continues for a longer period than in the absence of arsenate, and a greatly increased total fermentation results. The total amounts of carbon dioxide and alcohol produced therefore depend on the particular concentration of arsenate employed, and may be either higher or lower than that given by the juice in absence of arsenate.

Experiment 10 is a typical example of the phenomena observed when a moderate quantity of arsenate is used, while Experiment 11 exhibits the effect of variation of concentration of arsenate both on the rate and the total fermentation.

Experiment 10.—Parallel experiments were made with juice alone, and in the presence of arsenate, the solutions having the following compositions:—

1. 20 c.c. yeast-juice + 15 c.c. sodium bicarbonate + 5 c.c. arsenate.
2. 20 c.c. yeast-juice + 20 c.c. sodium bicarbonate.

Each solution contained 4 grm. of glucose.

Time.	1.		2.	
	Rate per 5 minutes.	Total evolved.	Rate per 5 minutes.	Total evolved.
h. m.	c.c.		c.c.	
15	9·5		2·1	
30	13		2·1	
45	14·2		1·9	
1	13·9	142·5	1·8	22·2
1 15	12·8		1·6	
1 30	11·9		1·5	
2	9·8	288	1·2	39·5
4	3·4	436	0·9	65·2
6	1·7	498	0·7	85
10	0·2	544	0·41	108
14	0	550	0·33	122
35			0	133

It will be seen that in the presence of arsenate (1) the maximum rate is attained in about 45 minutes, and amounts to 14·2 c.c. per 5 minutes; the total carbon dioxide evolved is 550 c.c., and fermentation ceases in about

12 to 14 hours. In the absence of arsenate (2), the maximum is attained at once and is 2·1, the total evolved is 133 c.c., and the duration of the fermentation is about 24 to 30 hours.

Experiment 11.—The totals evolved by equal volumes of the juice employed in the foregoing experiment were determined with various additions of arsenate, and are quoted along with the maximum rates and the approximate duration of fermentation.

No.	Arsenate added.	Maximum rate.	Total evolved.	Duration of fermentation.
	c.c.			hours.
1	0	2·8	133·3	24—30
2	0·5	25	1024	55—60
3	5	14·2	550	12—14
4	10	5·9	190·6	9—10
5	20	1·5	47·8	7

4 grm. of glucose were present in each case, and it is probable that fermentation in No. 2 came to a close on account of exhaustion of the sugar.

5. *The Fermentation of Mannose and Fructose by Yeast-juice in the Presence of Arsenate.*

The fermentation of mannose by yeast-juice is also accelerated by the addition of arsenate to about the same extent as that of glucose. In the case of fructose, however, a difference, similar in character to that previously noted in the presence of phosphate,* is also observed with arsenate, viz., the optimum concentration of arsenate is greater and the maximum rate attainable higher than in the case of glucose.

These facts are well brought out by the results of the following two experiments.

Experiment 12.—A series of different volumes of the standard arsenate solution were added to yeast-juice in presence of 1 grm. of the sugar in question, all the liquids being made up to the same volume, and the maximum rate attained in each case observed.

20 c.c. yeast-juice + x c.c. arsenate + $(26-x)$ c.c. of a solution containing 1 grm. of sugar.

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* Harden and Young, 'Roy. Soc. Proc.,' B, 1909, vol. 81, p. 336.

x.	Maximum rate for 5 minutes.		
	Glucose.	Mannose.	Fructose.
0	0·63	0·65	0·65
0·2	7·0		
0·5	9·1		11·7
1	9·8	8	14·4
2	9·7	6·6	16
4	4	4·5	14·8
8			8·4
16			2·4

Experiment 13.—A similar experiment was made with a mixture of 20 c.c. of yeast-juice and 2 grm. of the sugar, the total volume being 40 c.c.

Arsenate.	Maximum rate in 5 minutes.	
	Glucose.	Fructose.
c.c.		
0	1	1·2
0·2	7·2	
1	12·1	26·6
2	12·4	
3	13·4	
5	13·4	43·6
7		45·8
10		42·4
15	5·1	39

In this case the optimum concentration of arsenate in the presence of glucose is between 3 and 5 c.c., and the maximum rate is about 13·4, which is 13·4 times the normal rate. In the presence of fructose, on the other hand, the optimum concentration is about 7 c.c., and the maximum rate 45·8 c.c., which is 38·2 times the normal rate.

In Experiment 12 the juice evidently contains less fermenting complex, and there is not such a great disparity between the optima. The maximum rate for glucose is 9·8 c.c., for mannose, slightly less, 8 c.c., and for fructose, 16 c.c.

6. *Nature of the Effect of Arsenate on Fermentation by Yeast-juice.*

In considering the nature of the action of arsenate in accelerating the fermentation of sugars by yeast-juice two possibilities present themselves In the first place the arsenate may replace the phosphate in the main reaction :

$$(1)\quad 2C_6H_{12}O_6 + 2Na_2HPO_4 = 2CO_2 + 2C_2H_6O + 2H_2O + C_6H_{10}O_4(PO_4Na_2)_2.$$

The facts that much more than an equivalent of carbon dioxide is evolved at the accelerated rate and that the arsenate is found in the free state at every stage of the fermentation render it necessary to suppose that any arsenic compound which is formed is of a very unstable nature, and only exists at any moment in a very low concentration.

On the other hand, it is also possible that the arsenate accelerates the fermentation by increasing the rate at which free phosphate is produced from the hexosephosphate. This substance is hydrolysed by the enzyme hexosephosphatase according to the equation:

$$(2)\quad C_6H_{10}O_4(PO_4Na_2)_2+2H_2O = C_6H_{12}O_6+2Na_2HPO_4,$$

and it is the rate of this reaction which is the controlling factor of the rate of fermentation of sugar by yeast-juice in the absence of added phosphate.*

Acceleration of this change would therefore result in a constant increased supply of phosphate, and would enable reaction (1) to proceed at a correspondingly greater rate. In other words, the effect of the arsenate would be to produce a condition similar to that existing when free phosphate is added gradually to yeast-juice so that its concentration is maintained at the optimum, and the reaction proceeds at its maximum velocity as long as this condition is maintained.†

It also follows from the fact that the rate of fermentation is dependent upon the rate of production of free phosphate that the acceleration produced by the addition of arsenate cannot be due to an increase in the activity of the zymase alone. In a mixture of fresh yeast-juice and sugar this enzyme is not producing its maximum effect, as is shown by the fact that the addition of phosphate causes a large increase in the rate of fermentation. In other words there is an excess of enzyme already present in the mixture. An increase in the activity of the zymase, therefore, without an increase in the supply of phosphate, could not be expected to cause any acceleration in the rate of fermentation. It is possible, however, that the activity both of the hexosephosphatase and of the zymase might be simultaneously increased by the addition of arsenate. In this case it would be possible to obtain a higher maximum by the addition of arsenate in presence of the right amount of phosphate, than could be obtained by adding phosphate alone.

1. *Proof that Arsenate cannot replace Phosphate in the Fermentation of Sugars by Yeast-juice.*—The first of these alternative explanations, according to which arsenate is supposed to take the place of phosphate and to react with the sugar to form an unstable hexose-arsenate, can be investigated in

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* Harden and Young, 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 299.

† Harden and Young, 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 309.

the following way. A mixture of ferment and co-ferment can be prepared as previously described, which is free from phosphate and hexosephosphate,* and will not ferment sugar without the addition of phosphate. If now arsenate can replace phosphate, such a mixture should be rendered active by the addition of arsenate as well as by that of phosphate.

In order to realise these conditions zymin was freed from phosphates and co-ferment by repeated washing with water, until it was incapable of fermenting sugar. To this was added a solution of co-ferment, free from mineral phosphates and hexosephosphate, as previously described. This mixture was almost incapable of fermenting sugar, whilst the addition of phosphate, either in the form of mineral phosphate or hexosephosphate, readily caused fermentation. When, however, arsenate was substituted for the phosphate no fermentation ensued. This was not due to any inhibiting action of the arsenate, as in the presence of phosphate the same quantity of arsenate did not interfere with the fermentation.

Exp.	Zymin suspension.	Co-ferment solution.	Water.	Fructose.	Phosphate, 0·3 molar.	Hexose-phosphate.	Arsenate, 0·3 molar.	Gas evolved.			
	c.c.	c.c.	c.c.	grm.	c.c.	c.c.	c.c.	c.c.		hrs.	mins.
16. 1	10	20	1	2	0	0	0	7	in	24	
2	10	20	0	2	1·0	0	0	67	,,	24	
3	10	20	0·75	2	0	0	0·25	0·2	,,	2	
4	10	20	0	2	0	0	1	2·7	,,	24	
5	10	20	0·25	2	0·5	0	0·25	51·6	,,	24	
17. 1	10	20	6·3	0·7	0	0	0	1·5	,,	19	
2	10	20	0	0·7	0	6·3	0	132·7	,,	19	
3	10	20	6·3	0·7	0	0	0·5	1	,,	19	
4	10	20	0	0·7	0	6·3	0·5	133·5	,,	19	
18. 1	10	15	0	0·5	0	0	0·2	2·0	,,	20	
2	10	15	8·8	0·5	0	8·8	0	25·8	,,	1	50
3	10	15	0	0·5	0	8·8	0·2	47·8	,,	1	50

The hexosephosphate solution used was 0·21 molar for Experiment 17 and 0·14 molar in Experiment 18.

It will be seen that in no case was arsenate able to take the place of phosphate, and it must therefore be concluded that the action of arsenate is fundamentally different from that of phosphate.

Incidentally the foregoing experiments afford further evidence that phosphate is indispensable for the alcoholic fermentation of sugar. Comparing Nos. 1 and 2, Experiment 17, it is seen that in the almost complete absence of available phosphate only 1·5 c.c. of carbon dioxide were evolved, whereas in the presence of hexosephosphate 132·7 c.c. were obtained. The ratio of the totals produced in presence and in absence of phosphate is thus 132·7/1·5 = 88, a much greater value than had previously been observed.*

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* 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 322.

2. *Acceleration of the Action of Hexosephosphatase by Arsenate.*—The direct replacement of phosphate by arsenate being excluded, the alternative explanation, that the arsenate accelerates the action of the hexosephosphatase, remains to be considered. This question can readily be investigated experimentally, and the result of such an examination is to show that the arsenate does in fact produce a very marked effect of this kind, and that it is to this acceleration that the enhanced rate of fermentation in the presence of arsenate is due. This has been proved separately for yeast-juice and zymin.

In the three experiments carried out with yeast-juice, a comparison was made between the rates of fermentation in mixtures of yeast-juice with (*a*) hexosephosphate and arsenate; (*b*) water and arsenate; (*c*) hexosephosphate alone.

In experiments such as these, done in the absence of added sugar, the rate of fermentation is controlled by the production of sugar (1) from the hexosephosphate; and (2) from the glycogen and dextrins of the juice. Hence, by subtracting the rate of auto-fermentation in presence of arsenate (*b*) from the rate of fermentation of hexosephosphate also in presence of arsenate (*a*) a difference is obtained which represents the share of this enhanced rate which is due to the hydrolysis of the hexosephosphate. This is given by $a-b$, and a comparison of this with the rate of fermentation of hexosephosphate in absence of arsenate shows whether the effect of the arsenate has been to increase the rate of hydrolysis of the hexosephosphate. (In order to obtain the true rate of evolution due solely to the hexosephosphate in absence of arsenate the rate of auto-fermentation of the juice should be subtracted from that observed in presence of hexosephosphate (*c*). As, however, it is not certain what effect the presence of hexosephosphate may have upon the enzymic hydrolysis of glycogen, etc., this has not been done in the present instance. The effect of making this subtraction would be considerably to increase the cogency of the argument.)

In order as far as possible to diminish the auto-fermentation, the yeast-juice employed in Experiments 19 and 20 was incubated for some time before the addition of the hexosephosphate and water, and the yeast employed for the preparation of the juice for Experiment 21 was treated for the removal of glycogen by the method described by Buchner and Mitscherlich, but still yielded a juice which gave a considerable amount of auto-fermentation.

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* 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 324.

Experiments 19, 20, 21.—In each case 25 c.c. of yeast-juice + 0·5 c.c. of 0·3 molar arsenate + 20 c.c. of water or a normal solution of sodium hexosephosphate (containing 8·5 grm. of the acid per 100 c.c.) were employed, the liquids being made up to 45·5 c.c.

No. of experiment.	Time after addition of arsenate, in minutes.	Total c.c. evolved in the time *t*.			
		a. Arsenate + hexosephosphate.	*b.* Arsenate + water.	*a*−*b*.	*c.* Hexosephosphate + water.
19	15	40·5	26·2	14·3	6·3
	30	77·7	50·5	27·2	12·1
	75	147·9	90·6	57·3	22·3
	205	311·7	165·9	145·8	55·4

No. of experiment.	Time after addition of arsenate.	Rate in c.c. evolved in preceding 5 minutes.			
		a.	*b.*	*a*−*b*.	*c.*
	h. m.				
20	5	2·9	0·5	2·4	1·4
	10	3·7	0·6	3·1	0·9
	15	3·7	0·4	3·3	0·8
	20	3·7	0·5	3·2	0·9
	25	3·9	0·3	3·6	0·7
	30	3·5	0·4	3·1	0·7
	Total...	21·4	2·7	18·7	5·4
21	5	9·1	3·0	6·1	3·1
	10	8·6	2·3	6·3	2·9
	15	7·3	1·9	5·4	0·9
	15 20	6·8	1·2	5·6	1·2
	35	6·4	1·3	5·1	1·3
	40	6·9	1·7	5·2	1·6
	45	6·1	1·3	4·8	0·9
	50	5·6	1·0	4·6	0·8
	Total...	56·8	13·7	43·1	12·7

It will be seen that in every case the fermentation in presence of hexosephosphate and arsenate, less the auto-fermentation in presence of arsenate (*a*−*b*) is considerably greater (two to four times) than the rate of fermentation in absence of arsenate (*c*).

As this increased fermentation is carried on at the expense of the hexosephosphate, it follows that the hexosephosphate is being more rapidly decomposed than in the absence of arsenate, and that therefore free phosphate, which is also a product of the hydrolysis of this compound, must also be liberated in greater quantity. Since, moreover, hexosephosphate yields two equivalents of phosphate for one of sugar the phosphate must accumulate, and accordingly the rate of decomposition of the hexosephosphate can also be gauged by the rate of accumulation of free phosphate.

Experiment 22.—This has been ascertained by estimating the amount of free phosphate in the boiled and filtered solutions from Experiment 19. The following are the results, expressed in grams of $Mg_2P_2O_7$, the equivalent of the arsenate present having been subtracted in each case :—

(*a*)	Hexosephosphate + arsenate...............	0·6136
(*b*)	Auto-fermentation + arsenate	0·1144
(*c*)	Hexosephosphate without arsenate	0·0707

Hence, in presence of arsenate, $0·6136 - 0·0707 = 0·5429$ grm. has been liberated more than in its absence, and of this amount $0·5429 - 0·1144 = 0·4285$ grm. has been formed from the hexosephosphate which was added.

This result is therefore in entire agreement with the observations of the rates of fermentation.

Experiments with zymin confirm the conclusion that the arsenate accelerates the action of the hexosephosphatase in an equally striking manner. Zymin washed free from soluble phosphates and co-ferment was used, and a solution of co-ferment also almost free from available phosphate was added, together with an excess of fructose. This mixture was almost incapable of producing fermentation, owing to lack of phosphate, and as a matter of fact gave a total of only 1·5 c.c. of carbon dioxide, whereas when phosphate was added as much as 132 c.c. were involved. Solutions of hexosephosphate were added to two equal volumes of this mixture, and to one of these arsenate was also added. The sole source of phosphate in these two liquids was the hexosephosphate, and the rate of fermentation would therefore be controlled by the rate of production of phosphate from this. Any increase of fermentation in the presence of arsenate must therefore be due to the accelerating action of this salt on the hexosephosphatase.

Experiment 23.—The zymin and co-ferment solution were prepared in the same manner as those used in Experiment 17.

1. 10 c.c. zymin suspension + 15 c.c. co-ferment solution + 0·5 grm. fructose + 8·8 c.c. of 0·14 molar hexosephosphate + 0·2 c.c. arsenate.

2. The same mixture but containing 0·2 c.c. of water instead of arsenate.

Here the rate of fermentation, and consequently of hydrolysis of hexosephosphate, in presence of arsenate is about double that in its absence.

Time after addition of arsenate, in minutes.	C.c. evolved in preceding 5 minutes.	
	1.	2.
5	2·8	1·4
10	2·7	1·4
15	2·5	1·2
20	2·3	1·0
25	2·3	—
65	2·4	1·3
90	2·2	1·3
115	1·84	1·04
Total in 115..............	47·8	25·8

Whether the arsenate also accelerates the action of the zymase has not been decided with certainty. If this were so, the rate of fermentation attainable by the proper mixture of arsenate and phosphate would be greater than that given by phosphate alone. Since, however, the rate of fermentation in presence of phosphate falls off with diminishing concentration of this salt extremely rapidly and according to a law which has not yet been ascertained, it is impossible at present to institute an accurate comparison between the velocity constants of the two reactions. It may, however, be noted that cases have been observed in which the evolution of gas in five minutes was greater in presence of a certain quantity of arsenate than could be obtained with phosphate alone.

7. *Effect of Arsenate on the Auto-fermentation of Yeast-juice.*

When arsenate is added to yeast-juice which is undergoing auto-fermentation, a very considerable rise is produced in the rate of evolution of carbon dioxide; the rate attained is, however, much lower than that produced in presence of excess of glucose. The extent of this enhanced auto-fermentation is shown by the following (Nos. 1 and 2), whilst the effect of the same quantity of arsenate in presence of excess of glucose is given in columns 3 and 4.

Experiment 14.—Four solutions were incubated, all being made to the same volume of 30·5 c.c.

1. 20 c.c. yeast-juice + 10·5 c.c. water + 0 c.c. arsenate.
2. ,, ,, + 10 ,, + 0·5 ,,
3. ,, ,, + 9·3 ,, + 0 ,, + 2 grm. glucose.
4. ,, ,, + 8·8 ,, + 0·5 ,, + 2 ,,

The following rates were observed:—

	No. 1.	No. 2.	No. 3.	No. 4.
Initial rate per 5 minutes	1·5	8·5	2·5	36·5
Total in 1 hour 40 minutes	27·2	81·2		

The rate of auto-fermentation of yeast-juice is not, like the rate of fermentation of sugar, controlled by the rate at which phosphate is produced from the hexosephosphate, but by that at which sugar is formed by the hydrolysis of glycogen and dextrins, as is shown by the well known fact, confirmed in the foregoing experiment, that the rate of auto-fermentation is usually considerably less than that of the fermentation of sugar.

Consequently, any increase in the rate of auto-fermentation must be primarily due to an increased supply of sugar. This may be derived from two sources. A portion is supplied, as has been shown (Experiments 19–23), by the increased rate of hydrolysis of the hexosephosphate originally present in the juice. This continues until the whole of the sugar obtainable from this source has been converted into carbon dioxide and alcohol and has the effect of temporarily increasing the rate of fermentation. This acceleration is independent of the presence or absence of glycogen, and hence the effect of arsenate on the juice in the absence of added glycogen gives a superior limit to the extent of its influence.

After this point has been reached, the sugar formed by the hydrolysis of complex carbohydrates becomes the sole source of carbon dioxide. As fermentation then proceeds this supply also gradually becomes exhausted and the rate falls off slowly. In presence of added glycogen, on the other hand, the rate falls off more slowly.

In order, therefore, to ascertain whether the rate of hydrolysis of glycogen is increased by the addition of arsenate, it is necessary to determine whether the effect of adding glycogen and arsenate together to the juice is greater than the sum of the effects of adding glycogen and arsenate to separate portions of the juice.

This is done in the following experiment:—

Experiment 15.—Five quantities of 20 c.c. of yeast-juice were mixed with the following solutions and incubated for 1 hour 45 minutes, the total volume in each case being 30·5 c.c.—

1. 2·5 grm. glucose in 10·5 c.c. solution.
2. 10·5 c.c. water.
3. 10 c.c. water + 0·5 c.c. arsenate.
4. 0·5 grm. yeast-glycogen in 10·5 c.c. solution.
5. 0·5 grm. yeast-glycogen in 10 c.c. solution + 0·5 c.c. arsenate.

	1.	2.	3.	4.	5.
Initial rate (c.c. per 5 minutes)	2·5	1·5	8·5	1·9	10·0
Rate after 1 hour	—	1·4	2·9	1·6	7·2
Rate after 1 hour 40 minutes	—	1·1	1·85	1·35	4·45
Total evolved in 1 hour 45 minutes	—	27·0	81·2	32·4	141·2

It is to be noted, in the first place, that the rate in presence of glycogen (1·9) is less than in presence of sugar (2·5), so that the rate of fermentation in No. 4 is actually controlled by the rate of production of sugar.

The effect of adding glycogen to the juice increases the total by (4)–(2) .... 32·4 − 27 = 5·4 c.c.

The effect of adding the arsenate to the juice alone increases the total by (3)–(2) .... 81·2 − 27 = 54·2 „

Sum of the separate effects of glycogen and arsenate .... 54·2 + 5·4 = 59·6 „

Effect of adding glycogen and arsenate together (5)–(2).................................... 141·2 − 27 = 114·2 „

Hence at least 114·2 − 59·6 = 54·6 c.c. more carbon dioxide have been derived from glycogen in the presence of arsenate than in its absence. Comparing the rates per five minutes given in columns 3 and 5, showing the effect of arsenate on the fermentation in absence and in presence of added glycogen, it is to be noticed that the initial rates are not greatly different (8·5 and 10). This is due to the fact that at the commencement of the auto-fermentation there is an ample supply of glycogen. This, however, soon begins to fail, and consequently the rate falls off more rapidly than in the presence of added glycogen, the relative rates after 1 hour being 2·9 and 7·2, and after 1 hour 40 minutes, 1·85 and 4·45. For this reason the comparison is best made between the totals evolved after some time and not between the initial rates.

Further evidence in support of the conclusion that the hydrolysis of glycogen is accelerated by the addition of arsenate is afforded by the effect of the addition of glycogen to Solutions 2 and 3 in the foregoing experiment, which had been allowed to auto-ferment in absence and presence of arsenate respectively.

	No. 2.	No. 3.
5 minutes' rate before addition of glycogen	1·1	1·85
,, ,, after	1·4	4·4

Even this enhanced rate of glycogen hydrolysis is not nearly rapid enough to supply sufficient sugar for the fermenting complex in presence of arsenate. The maximum rate obtained with glycogen was 10, whereas the addition of 0·5 c.c. of arsenate to Solution 1 containing excess of glucose produced a rate of 36·5 c.c. in five minutes.

It follows from the foregoing that arsenate has the power of greatly accelerating the action of the diastatic enzyme in yeast-juice.

8. *Action of Arsenite on the Fermentation of Sugar by Yeast-Juice.*

Buchner* made a number of observations on the effect of arsenite on the fermentation of sugars by yeast-juice. The presence of 2 per cent. of arsenious oxide, in the form of the potassium salt, was found in some cases completely to inhibit the fermentation of glucose, but not of saccharose, its effect on fructose being of an intermediate character, whilst, in other cases, the same concentration caused a marked increase in the total fermentation of all three sugars.

A powerful inhibitory effect was also produced by this concentration of arsenite, even on the fermentation of saccharose by (*a*) juice which had been prepared from yeast which had been kept at 8° for three days, (*b*) juice which had been dialysed against water for 16 hours, (*c*) juice which had been evaporated at 35°, (*d*) juice diluted with one volume of water.

It was further observed, in many cases, that the presence of arsenite caused a considerable increase in the fermentation during the first 16 hours, even when the total fermentation was less than in the absence of arsenite. It was found that this inhibitory action was not due to the conversion of the arsenite into arsenate; and, further, that it was lessened by the addition of heated yeast-juice or blood serum, either heated or unheated. The inhibitory action, moreover, was greater when the arsenite was incubated with the juice in the absence of added sugar. Buchner advanced no explanation of the preliminary acceleration caused by arsenite, and regarded the inhibitory effect as due to some change in the colloidal state of the enzyme, against which the presence of other colloidal substances partially protected it. It was, according to this, the diminution in the amount of these protecting colloids which made the samples of juice submitted by him to various operations so much more susceptible to the action of arsenite.

A careful experimental comparison of the effects of arsenite and arsenate on fermentation by yeast-juice shows that these two actions are precisely similar in kind, but that the acceleration produced by arsenite is markedly less than that caused by an equivalent concentration of arsenate. This similarity of the action of arsenite to that of arsenate is shown in the experiments summarised below.

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* 'Die Zymasegärung,' pp. 184—205.

(1) Arsenite produces an acceleration which continues long after an extra amount of carbon dioxide equivalent to the arsenite added has been evolved.

Experiment 24.—Yeast-juice containing 10 grm. of glucose per 100 c.c. was incubated until a steady rate of fermentation had been attained. To 20 c.c. of this were added 5 c.c. of a 0·3 molar solution of sodium arsenite, equivalent to 33·6 c.c. of carbon dioxide at N.T.P., and the rate of fermentation observed.

Time after addition, in minutes.	Rate in c.c. of carbon dioxide evolved in preceding 5 minutes.	Total.	Time after addition, in minutes.	Rate in c.c. of carbon dioxide evolved in preceding 5 minutes.	Total.
0	1·7		25	6·2	32·8
5	7·0	7·0	30	5·9	38·7
10	7·1	14·1	45	5·6	56·5
15	6·2	20·3	60	5·6	73·2
20	6·3	26·6	85	3·3	89·7

The total evolved in 85 minutes is thus 89·7 c.c., whereas in absence of arsenite it would have been 17 × 1·7 = 28·9, and hence the extra carbon dioxide is 89·7 − 28·9 = 60·8, which is largely in excess of the equivalent, 33·6.

(2) The acceleration produced by arsenite is much less than that obtained with an equivalent amount of arsenate.

Experiment 25.—20 c.c. of yeast-juice, containing 2 grm. of glucose, gave a rate of 1·7 c.c. in five minutes. The addition of 1 c.c. of a 0·3 molar solution of arsenite raised the rate to 3·1 c.c. in five minutes, whilst the addition of the same volume of 0·3 molar arsenate to the original juice gave a rate of 27·5 c.c.

(3) The rate attained increases with the concentration of the arsenite until a maximum is reached, so that, as in the case of arsenate, there is an optimum concentration of arsenite.

Experiment 26.—Varying quantities of a 0·3 molar solution of sodium arsenite were added to 20 c.c. of yeast-juice, the total volume being maintained at 40 c.c., and containing 10 per cent. glucose.

(4) The maximum rate attainable and the optimum concentration of arsenite are both greater in the case of fructose than in that of glucose.

C.c. arsenite added.	Rate in c.c. of carbon dioxide in 5 minutes.
0	2·1
1	3·4
3	6·4
5	6·9
13	4·5
20	0·8

Experiment 27.—The numbers in the following table refer to 20 c.c. of yeast-juice and 1 grm. of the sugar in 35 c.c. total volume.

C.c. molar arsenite added.	Rate in c.c. per 5 minutes.	
	Glucose.	Fructose.
0	0·34	0·5
2·5	1·17	1·45
5	2·7	3·5
10	2·7	7·96
15	1·48	9·8

It will be noted that the optimum concentration was not attained for fructose, whilst for glucose it was between 5 and 10 c.c.

(5) When a mixture of ferment, co-ferment, and sugar is rendered so free from phosphate that no fermentation will take place without the addition of phosphate, this added phosphate cannot be replaced by arsenite. Arsenite therefore cannot exercise the same function in alcoholic fermentation as phosphate.

Experiment 28.—The following experiment was carried out in a similar manner to Experiment 17 dealing with arsenate.

To equal volumes of a mixture of 10 c.c. of washed zymin suspension + 20 c.c. co-ferment solution free from phosphate + 0·22 grm. of fructose were added the following:—

		C.c. CO_2 in 18 hours.
1.	6·5 c.c. water	1·5
2.	6 „ + 0·5 c.c. phosphate	38
3.	6 „ + 0·5 „ arsenite	1·4
4.	6 c.c. normal hexosephosphate + 0·5 c.c. arsenite	33

The result of (4) shows that the amount of arsenite present was not sufficient to inhibit the action of the enzyme, whilst (3) shows that arsenite alone produced no increase in fermentation.

(6) Arsenite accelerates the rate of hydrolysis of glycogen, and hence the auto-fermentation of yeast-juice.

Experiment 29.—The following solutions were incubated and the rates of fermentation observed :—

1. 20 c.c. of yeast-juice from well-washed yeast + 14 c.c. water.
2. 20 c.c. of the same juice + 9 c.c. water + 5 c.c. of 0·3 molar arsenite.

Experiment 30.—In this case 25 c.c. of yeast-juice were used along with (1) 15 c.c. of water; (2) 10 c.c. water and 5 c.c. of 0·3 molar arsenite.

Experiment.	Time, in minutes.	Auto. + water.		Auto. + arsenite.	
		Rate in 5 minutes.	Total.	Rate in 5 minutes.	Total.
29	5	0·6	0·6	2·6	2·6
	10	0·5	1·1	2·2	4·8
	15	0·8	1·9	2·2	7·0
	20	0·5	2·4	2·4	9·4
	60	0·55	6·8	2·6	26·7
30	5	0·9	0·9	4·5	4·5
	10	1·0	1·9	4·0	8·5
	15	1·0	2·9	—	—
	20	1·4	4·3	3·8	16·1
	60	1·3	9·8	4·3	33·5

That this increase is, at all events in part, due to an acceleration of the rate of hydrolysis of glycogen is shown in precisely the same manner as for arsenate (Experiment 15). The results obtained are as follows :—

Experiment 31.—Five quantities of 25 c.c. of yeast-juice were incubated with the following solutions and the fermentations observed :—

1. 0·5 grm. glucose in 15 c.c. solution.
2. 15 c.c. water.
3. 10 c.c. water + 5 c.c. of 0·3 molar arsenite.
4. 0·5 grm. glycogen in 15 c.c. solution.
5. 0·5 grm. glycogen in 10 c.c. solution + 5 c.c. arsenite.

	1.	2.	3.	4.	5.
Initial rate (c.c. per 5 minutes) ...	3·0	1·3	4·0	1·6	4·2
Total evolved in 5 hours	—	67·8	118·3	73·7	152·1

Here, as in the experiment with arsenate, the rate in presence of glycogen (1·6) is less than that in presence of glucose (3·0), so that the rate of the fermentation in (4) is actually controlled by the rate of production of sugar.

The effect of adding glycogen to the juice is to increase the total by	73·7−67·8 = 5·9
The effect of adding the arsenite alone to the juice is to increase the total by	118·3−67·8 = 50·5
Sum of separate effects of glycogen and arsenite ...	50·5+ 5·9 = 56·4
Effect of adding glycogen and arsenite together is	152·1−67·8 = 84·3

Hence at least 84·3−56·4 = 27·9 c.c. more carbon dioxide have been derived from the glycogen in the presence of arsenite than in its absence in the same time.

(7) Arsenite produces its effect by accelerating the action of the hexosephosphatase.

Experiment 32.—The experiments on this point were carried out exactly as described under arsenate (Experiments 19, 20, 21), but, in addition, the auto-fermentation of the juice (*d*) was observed—

t.	Total c.c. evolved in time *t*.					
	a.	*b*.	*c*.	*d*.	*a*−*b*.	*c*−*d*.
5	4·1	2·6	1·0	0·6	1·5	0·4
10	7·7	4·8	2·2	1·1	2·9	1·1
15	11·2	7·0	3·4	1·9	4·2	1·5
20	14·8	9·4	4·6	2·4	5·4	2·2
25	17·9	11·7	5·6	2·8	6·2	2·8
55	35·7	24·2	10·7	6·2	11·5	4·5
60	38·7	26·7	12·3	6·8	12·0	5·5
65	41·7	28·8	13·2	7·3	12·9	5·9

As already explained, the original fermentation due to hexosephosphate alone is given by *c*−*d*, and the enhanced fermentation due to hexosephosphate by *a*−*b*. The effect of arsenite on the hexosephosphatase is very much less than that of arsenate, as was to be expected from the relative effects of the two on the fermentation of sugar, and is only clearly visible when the original auto-fermentation is subtracted from the fermentation in presence of hexosephosphate. The result in this case therefore depends on the assumption that the presence of the hexosephosphate and the products of its hydrolysis does not seriously affect the rate of hydrolysis of the glycogen.

Experiment 33.—An experiment was also carried out with washed zymin in precisely the same way as described for arsenate (Experiment 23), hexosephosphate being made the sole source of phosphate.

10 c.c. of washed zymin suspension+15 c.c. of co-ferment solution free

from phosphate + 0·5 grm. of fructose + 9 c.c. of hexosephosphate solution + 1 c.c. of water, on addition of—

(1) 1 c.c. water gave 9·5 c.c. of carbon dioxide in 25 minutes.

(2) 1 c.c. of 0·3 molar arsenite solution gave 11·1 c.c. in 25 minutes.

In this case the rate was only increased about 20 per cent., whereas the addition of arsenate doubled the rate.

9. *Inhibitory Action of Arsenate and Arsenite.*

Both arsenate and arsenite may exert an inhibitory action on alcoholic fermentation, and this may manifest itself in two ways. If a sufficient excess be added, no fermentation at all occurs; in presence of a smaller amount than is necessary to produce this total inhibition, the effect is manifested by the low rate and shortened duration of fermentation. An experiment showing this behaviour of arsenate in a typical manner has already been quoted (No. 11). Even when the rate of fermentation is considerably increased by the presence of an amount of arsenate in excess of the optimum amount, it frequently happens that this rate falls very rapidly and that all fermentation ceases in an hour or two. The exact conditions under which this occurs have, however, not been ascertained. It is as yet uncertain whether this inhibitory action is due, as suggested by Buchner, to changes in the colloidal state of the fermenting complex or to the formation of inactive combinations of the complex with the arsenate and arsenite, in a similar manner to that suggested by the authors in the case of inhibition produced by excess of phosphate.* In all probability the phenomenon is a complex one in which several factors participate.

The total inhibition produced by arsenite which was observed by Buchner (p. 469) with juice which had been submitted to dialysis, evaporation, etc., may not improbably be ascribed to a loss of fermenting complex during these operations, so that the amount of arsenite added was greatly in excess of the optimum. It is, however, quite possible that the proteins accompanying the ferment present in the juice do exert a protective influence, as suggested by Buchner, and that a diminution in their amount may also play some part in the phenomenon.

10. *Experiments with Various Salts.*

No other salts capable of bringing about results in any way comparable with those produced by arsenates and arsenites have been found. Experiments with the sulphate, orthovanadate, antimonate, tungstate, chromate, stannate, phosphite, and citrate of sodium, and with tartar emetic and zinc sulphate, were all negative.

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* 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 299.

Summary.

1. When a suitable amount of arsenate is added to a fermenting mixture of yeast-juice and a sugar, it causes a large acceleration in the rate of production of carbon dioxide and alcohol. This enhanced rate differs from that produced by phosphate, inasmuch as it continues long after a chemical equivalent of carbon dioxide has been evolved. The arsenate, moreover, is found in the free state throughout the fermentation.

2. The rate attained increases rapidly with addition of arsenate, until an optimum concentration is reached, after which it decreases, at first rapidly, and then more slowly.

3. The total fermentation produced depends on the particular concentration of arsenate employed, and may be either higher or lower than that given in the absence of arsenate. As the high rate produced by a suitable quantity of arsenate persists for a long time, very considerable increases in the total fermentation may be observed.

4. Glucose and mannose are similarly affected by yeast-juice in presence of arsenate, whereas fructose is much more rapidly fermented than these two sugars, and the optimum concentration of arsenate in its presence is greater.

5. The increased rate of fermentation of sugars in presence of arsenate is due to an acceleration of the rate of action of the hexosephosphatase of the juice, whereby an increased supply of phosphate is afforded. The action is therefore essentially different from that of phosphate, and it has been found that arsenate cannot replace phosphate in the fundamental reaction of alcoholic fermentation.

6. Arsenate also causes a considerable increase in the rate of the autofermentation of yeast-juice, and in the rate of the fermentation of glycogen. This is mainly due to an acceleration of the rate of action of the diastatic enzyme of yeast-juice (glycogenase).

7. The action of arsenites is similar to that of arsenates, but is much less marked.

8. Both arsenate and arsenite cause total inhibition of the fermentation when they are present in a high concentration, but the nature of this effect has not been ascertained.

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Über die Zusammensetzung der durch Hefepreßsaft gebildeten Hexosephosphorsäure. I.

Von

A. Harden und W. J. Young.

(Aus dem Biochemical Department of the Lister Institute London.)

(Eingegangen am 22. März 1911.)

Im Jahre 1905 zeigten die Verfasser¹), daß, wenn man zu einem Gärungsgemisch, aus Glucose und Hefepreßsaft bestehend, ein lösliches Phosphat zusetzt, die Gärung bedeutend aktiviert wird. Diese Erscheinung ist aber nur vorübergehend, die Gärung erreicht bald eine konstante Größe, die nur wenig höher ist als die Gärung des ursprünglichen Gemisches ohne Phosphat. Es wurde gezeigt, daß am Ende der Periode der vermehrten Gärung das Phosphat nicht mehr durch Magnesiumcitratgemisch gefällt werden konnte. In einer Serie von späteren Arbeiten²) kamen die Verfasser zu der Ansicht, daß es sich um eine Bildung eines Phosphorsäureesters handelt. Die Reaktion kann durch folgende Gleichung ausgedrückt werden:

$$2\,C_6H_{12}O_6 + 2\,R'_2HPO_4 = 2\,CO_2 + 2\,C_2H_6O + C_6H_{10}O_4(PO_4R'_2)_2 + 2\,H_2O.$$

Ähnliche Erscheinungen wurden auch bei der Gärung von Fructose und Mannose unter denselben Bedingungen beobachtet.

Im Jahre 1907 erschien nun eine Arbeit von Iwanoff³), in der er angibt, daß während der Gärung von Glucose und Fructose durch Zymin, in Gegenwart von Natriumphosphat, organische Phosphorverbindungen entstehen, die in Form von Kupfersalzen isoliert

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¹) Proc. of the Chem. Soc. 21, 189, 1905.

²) Proc. Roy. Soc. B. 77, 417, 1906; 80, 299, 1908; 81, 336, 1909; Proc. of the Chem. Soc. 24, 115, 1908.

³) Zeitschr. f. physiol. Chem. 50. 281, 1907.

wurden. Seiner Meinung nach waren es Verbindungen einer Triose und Phosphorsäure, und er erhielt mit Phenylhydrazin zwei Osazone, die bei 142° resp. bei 125° schmolzen. In einer späteren Mitteilung¹) kam Iwanoff zu der Ansicht, daß beide Osazone identisch waren, und daß beide nach dem Umkrystallisieren aus Benzol bei 127° schmolzen. Die Analyse zeigte, daß es sich um ein Trioseosazon handelte. Er betrachtet deswegen diese Verbindung als eine Triosephosphorsäure. Im Jahre 1907 beschrieb Young²) die Isolierung dieser Phosphorverbindungen mit Hilfe der Bleisalze, deren empirische Zusammensetzung $C_3H_5O_2(PO_4Pb)$ war. v. Lebedew³) beschreibt ebenfalls die Isolierung dieses Phosphorsäureesters mit Hilfe von Aceton und findet, daß diese Verbindung mit Phenylhydrazin ein Osazon von der Formel $C_{24}H_{29}O_6N_6P$ liefert, die er als ein Pheny-l hydrazid des Phosphorsäurederivats des Hexosazons betrachtet.

Im selben Jahr wurden von Young⁴) die Eigenschaften der freien Hexosephosphorsäure (durch Gärung von Glucose, Fructose und Mannose in Gegenwart von Phosphat erhalten) sowie ihrer Salze eingehend beschrieben und die Meinung ausgesprochen, daß die Verbindungen der 3 Hexosen identisch sind und Salze der Säure $C_{16}H_{10}O_4(PO_4H_2)_2$ sind. Es wurden Silber-, Blei-, Barium- und Calciumsalze hergestellt, deren Zusammensetzung auf die obige Formel paßt.

v. Lebedew⁵) gibt in einer späteren Arbeit weitere Experimente über das Osazon und einige seiner Derivate an. Das umkrystallisierte Osazon schmolz bei 150 bis 152° und entsprach der Formel $C_{24}H_{31}N_6O_7P$, die ein wenig von der früher angegebenen differiert. Er hält es für ein phenylhydrazidophosphorsaures Derivat des Hexosazons und schreibt ihm folgende Zusammensetzung zu:

$$C_6H_8N_2H_2PO_4 . C_4H_5(OH)_3 . C(NNHC_6H_5)CH . NNHC_6H_5 .$$

v. Lebedew gibt ferner an, daß er ein Phosphorsäurehydrazid nicht erhalten konnte, somit konnte freie Phosphor-

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¹) Centralbl. f. Bakt., II. Abt., 24, 1, 1909.

²) Proc. of the Chem. Soc. 23, 65, 1907.

³) Diese Zeitschr. 20, 114, 1909.

⁴) Proc. Roy. Soc. B 81, 528, 1909.

⁵) Diese Zeitschr. 28, 213, 1910.

säure während der Bildung des Osazons nicht abgespalten werden. Das müßte aber der Fall sein, wenn der Phosphorsäureester zwei Phosphorsäurereste auf eine Hexosemolekül enthalten würde, der Formel von Young entsprechend. Deshalb kommt er zum Schluß, daß Hexosephosphorsäure nur einen Phosphorsäurerest enthält und erklärt die Tatsache, daß die Analysenresultate der Salze mit der Formel von Young zusammenstimmen dadurch, daß es sich um amorphe Substanzen handelt, die durch ein Phosphor enthaltendes kolloidales Polysaccharid verunreinigt sind (dessen Gegenwart er im Hefepreßsaft annimmt), das durch Bleiacetat gefällt sein soll. Diese Verbindung kann nach v. Lebedew durch Ultrafiltration aus dem Hefepreßsaft entfernt werden und liefert sofort kein Osazon, dagegen ein Phosphor enthaltendes Osazon nach 3 stündigem Kochen mit 2,5 normaler Schwefelsäure.

v. Lebedew zweifelt demnach an der Richtigkeit der Gleichungen der Verfasser, die den Kreislauf der Phosphorsäure in der Gärung darstellen und die auf der Formel $C_6H_{10}O_4(PO_4R_2)_2$ (die für Hexosephosphat angenommen wurde) beruhen.

$$\text{I.}\quad 2\,C_6H_{12}O_6 + 2\,R'_2HPO_4 = 2\,CO_2 + 2\,C_2H_6O + C_6H_{10}O_4(PO_4R'_2)_2 + 2\,H_2O.$$

$$\text{II.}\quad C_6H_{10}O_4(PO_4R'_2)_2 + 2\,H_2O = C_6H_{12}O_6 + 2\,R'_2HPO_4.$$

Abgesehen von der Zusammensetzung von Hexosephosphat wurde die Gleichung I ermittelt aus der Bestimmung: erstens, des Verhältnisses zwischen dem zugesetzten Natriumphosphat und CO_2 und Alkohol, die gebildet wurden als Folge dieses Zusatzes in Gegenwart eines Überschusses an Zucker, zweitens, aus dem Verhältnis von verbrauchtem Zucker und entstandener CO_2, in Gegenwart eines Überschusses an Phosphat.

Die ausgeführten Bestimmungen¹) setzten außer Zweifel, daß: erstens in der Periode der aktivierten Gärung, in Gegenwart eines Zuckerüberschusses und nach Zusatz von 1 Mol. Phosphat, die Reaktion nach der Gleichung I verläuft und 1 Mol. CO_2 und 1 Mol. Alkohol liefert und daß: zweitens, in Gegenwart eines Überschusses an Phosphat 1 Mol. CO_2 aus 1 Mol. Zucker gebildet wird. Die einzige Gleichung, die mit diesen

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¹) Proc. Roy. Soc. B. 77, 405, 1906; 82, 321, 1910.

experimentellen Daten und mit der Bildung eines Phosphorsäurederivats mit 6 Kohlenstoffatomen in Einklang steht, ist die oben angegebene Gleichung I. Diese Ansicht findet außerdem eine Stütze in den Analysenresultaten der Salze von Young. Die ganze Frage der Zusammensetzung der Hexosephosphorsäureester und ihrer Derivate, die durch Einwirkung von Phenylhydrazin erhalten wurden, wurde durch Young einer erneuten Revision unterworfen, und die erhaltenen Resultate werden in der folgenden Arbeit beschrieben. Es möge hier angeführt werden, daß sie vollständig die früher angenommene Formel der Hexosephosphorsäure bestätigen. Wir sind daher nicht in der Lage, die Stichhaltigkeit der Lebedewschen Argumente gegen die von uns aufgestellte Gleichung anzuerkennen.

Reprinted from the British Medical Journal, *November 5th, 1910.*

ON THE STRUCTURE OF COMPLEMENT IN RELATION TO DEVIATION.

Contribution to a Discussion in the Sections of Pathology and Bacteriology at the Annual Meeting of the British Medical Association. London, July, 1910.

BY

J. HENDERSON-SMITH, M.B.,
Assistant Bacteriologist to the Lister Institute.

The mechanism of complement deviation has already been so clearly explained by Professor Wassermann and Professor Muir in the course of this discussion that I need not again describe the process in any detail. Red blood corpuscles when treated or "sensitized" with the serum of an animal immunized to them acquire the power of attracting complement, a substance found in fresh normal serum. This is a property which the red corpuscles share with other cells similarly treated. Most cells, including bacteria, have the property of attracting complement, when they are combined with the serum of an animal immunized to them. On this fact is based the whole technique of complement deviation as a means of diagnosis.

Now this attraction of the sensitized red cells for complement may be accentuated or diminished according to circumstances which are within our control. It is a familiar fact that at a low temperature the sensitized red cells are usually unable to take up complement out of the the fluid in which they are suspended; they consequently remain unlaked. Similarly, if the complement is offered to the sensitized cells in a fluid which contains a high concentration of sodium chloride—that is, is hypertonic—their capacity of taking up the complement may be considerably reduced. There are other methods known, by which we can inhibit these sensitized cells from obtaining the complement offered to them; but the one to which I wish to direct your attention is that of altering the reaction of the medium in which the haemolytic constituents are mixed.

In a mixture of a definite slight degree of acidity haemolysis does not occur. The red cells are present sensitized, but they cannot avail themselves of the complement which is also present, and they remain unlaked.

[545/10]

It was shown by Michaelis and Skwirsky that under such conditions the red cells, although not laked, may still be not unaffected by the complement with which they have been in contact. The corpuscles are suspended in an isotonic solution of acid sodium phosphate of known acidity, and mixed with complement and the appropriate immune serum. After an interval the mixture is centrifugalized, and the supernatant fluid is removed from the unlaked red cells, and both fluid and red cells neutralized. Even after suspension in a neutral fluid the red cells remain unlaked. They are fully sensitized, because the addition of fresh complement causes rapid laking. The failure of haemolysis, then, is evidently due to the fact that the sensitized cells have not taken up the complement when it was offered them.

We should expect, then, to find the complement left in the supernatant fluid, and available in the now neutralized fluid. If, however, we add this neutral fluid to other red cells, sensitized equally, but in the usual way, by the addition of immune serum without complement, no haemolysis occurs. We do not, in fact, find the complement in the supernatant fluid. What, then, has become of it? An explanation, which immediately offers itself, is that the complement has been destroyed by the acidity of the original mixture. This, however, is not the case. For, if one adds the neutralized fluid to the cells from which it was separated by means of the centrifuge while it was acid, haemolysis occurs as readily as if we added fresh untreated complement. The complement has not been destroyed.

We are then in this case. We have a fluid, the neutralized supernatant fluid, which is unable to lake cells sensitized in the ordinary way. This fluid, then, must lack an ingredient essential to haemolysis, namely, the complement or part of it. It can, however, lake the cells from which it was removed. These cells, then, must contain the missing ingredient. But we know that these cells do not contain the complete complement. Hence we may deduce that the cells contain one fraction of the complement, which I shall call C, and the supernatant fluid another fraction, C′. The argument may be summarized in this way:

1. Complement + ordinarily sensitized cells = laking.
   Supernatant fluid + ordinarily sensitized cells = no laking.
      Therefore the supernatant fluid does not contain the complete complement.
2. Treated sensitized cells by themselves = no laking.
      Therefore these cells do not contain the complete complement.
3. Supernatant fluid + treated sensitized cells = laking.
      Therefore this mixture contains the complete complement.

   That is—The treated cells contain one fraction of the complement C, and the supernatant fluid another fraction of the complement C′.

A similar division of complement into two parts was effected some years ago by Ferrata by means of dialysis; but his method is very uncertain and gives very irregular results. One portion came down with the globulins which fell out of solution as dialysis progressed, and the other portion remained in solution. Ferrata believed that the globulin part, which corresponds to what we are calling C, is thermostable. But I find that not only is C′ destroyed by half an hour at 55° C., but also C. For, if you heat whole complement, you cannot by adding C′ restore its activity. It is interesting to note that a C derived from human serum by the acid method described acts at least as well with the C′ of guinea pig serum as does guinea-pig C itself. I am not yet certain whether the C′ of human serum acts with the C of the guinea-pig

Now, in deviation experiments, as ordinarily carried out—that is, with extracts, whether of bacteria or of syphilitic liver, as in the Wassermann reaction—it is not the whole of the complement that is deviated. After completing a strongly positive Wassermann reaction or a deviation with bacterial extract, the corpuscles settle down in the tube unlaked, and the supernatant fluid remains clear and untinted with haemoglobin. Such a supernatant fluid is incapable of laking red cells highly sensitized in the ordinary way, but, if you add it to cells treated in the manner described with acid phosphate, laking occurs quickly. The supernatant fluid therefore contains C′, and only part of the complement has been deviated—namely, C. In deviation experiments, then, what occurs is the removal of C, while C′ remains free and intact. I have tried this with various forms of deviation, such as the ordinary bacterial extract, the Wassermann reaction, and the precipitin reaction, and always with the same result: only C is deviated. Even in cases of spontaneous deviation, those non-specific deviations of complement which render rigid controls so necessary a part of a reliable Wassermann technique, it is not a deviation of the whole complement that occurs, but of only a part, and of always the same part—namely, C.

Whether if one used, instead of bacterial extracts according to the modification introduced by Wassermann, the whole bacteria as in the original method of Bordet and Gengou, one would still find only a part of the complement deviated, I cannot yet say. The attraction of red cells sensitized under ordinary conditions is for the whole complement, since they undergo lysis. It is reasonable to expect that in the case of bacterial cells the same will hold good. At least this expectation is reasonable for the bacteria, such as cholera or typhoid, which undergo lysis under the action of complement and immune serum. It will be of considerable interest, theoretical and practical, to determine whether organisms, such as many cocci, which do not undergo lysis, take up the whole complement or remain undissolved, because they are capable of taking up only a part, the portion corresponding to C.

Dr. J. C. G. Ledingham's Report to the Local Government Board on the Enteric Fever "Carrier"; being a review of current knowledge on this subject.

N° 30

ARTHUR NEWSHOLME,
Medical Officer,
5th October, 1910.

PREFACE.

Although the discovery of the "carrier"* as an agency in the transmission of enteric fever is a comparatively recent event, the literature of this subject has already attained large dimensions. This result is due to more than the interest created by the detection of a previously unrecognised channel of transference of the disease; it is, in great part, owing to the belief that the carrier is more than a mere transmitter of infection.

In the earlier days of bacteriology, with increasing bacteriological knowledge had come increasing difficulty in understanding how and where the infective organism of enteric fever maintained its vitality between the recognised occurrences of the disease, whether in the form of epidemic outbreaks or in that of endemic prevalence, to which it gave rise. Some degree of explanation, no doubt, was to be found in the probable existence of unrecognised cases of the fever, which might serve to secure the life-chain of the organism, but this hypothesis was not regarded as sufficient in itself. It came, accordingly, to be considered probable that the organism led a saprophytic existence outside the human body, and was capable of living and multiplying in such inanimate media as water and soil. This hypothesis at first received considerable support from bacteriologists, but as the result of further investigations fell largely into discredit. Following on this, came the discovery of the carrier, and with it the view that the

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* A "carrier" of enteric fever is a person who, although he may be in good health, carries the infectious material of the fever in his body, from which it may be given off in the stools or in the urine.

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carrier of enteric fever is not, like inanimate agencies, a merely passive transmitter of infection, but is a breeding ground and storehouse of the specific organism of the fever. These organisms live and flourish in his body, from which they may, whether continuously or from time to time, be excreted and give rise to the disease in others.

It appears, therefore, that not only persons sick with the fever, but also healthy persons who are carriers may infect others. It is contended that, by these two cycles of infection, the continued existence and infectivity of the enteric fever organism can be largely, if not wholly, explained without need for assumption of long viability of the organism outside the human body.

A hypothesis of this importance naturally attracted much attention and led to many investigations of the question. The results of these investigations are to be found in numerous publications by the observers who, in different countries, have been working in this field. But to obtain access to these widely scattered publications is a matter of no small difficulty, and accordingly Dr. Ledingham, who, along with myself, is engaged, on behalf of the Board, in an inquiry into protracted and recurrent infection in enteric fever, considered that there would be advantage in preparing for publication a summary of at least the more important investigations that have hitherto been made in connection with this subject.

In the accompanying report by Dr. Ledingham a summary of the sort is given. A considerable part of the report is devoted to the literature of the question of principal importance, that, namely, of the infectivity of carriers. Dr. Ledingham gives the history of a large number of occurrences of enteric fever in domestic life, in institutions, and in military populations, in which the source of infection has been traced, more or less convincingly, to a carrier. In many of the examples that he quotes, the evidence incriminating the carrier is conclusive. The infection was conveyed from the carrier to his victim, in many instances, by food or milk, while, in not a few others, the personal relationship between the carrier and the person attacked was sufficiently close to suggest direct personal infection. What share of enteric fever is directly due to the carrier is a problem which has been investigated by several observers, and the results of their investigations vary markedly. The proportions recorded by different inquirers range between 2·3 per cent. and 32 per cent. of the total amount of the fever. Some of those who have obtained the higher figures maintain that their results were due to more careful and sustained investigation and are the more accurate.

In view of the potential danger of the carrier to the community in which he lives, it is of interest to know what proportion of these persons exists. In this connection, Dr. Ledingham sets out the results of investigations undertaken with a view to ascertaining the degree of frequency with which carriers, whether those in whom the condition lasts but a short time (transitory carriers), or those in whom it is more permanent (chronic carriers), have been found in certain groups of persons. These investigations were made in relation with persons who had recently had enteric fever, with persons known to have been in contact with cases of the fever, and with an ordinary population. The results show considerable variations, perhaps partly dependent upon the differing degree of frequency with which bacteriological examinations were made and on the varying degree of efficiency of the methods employed. Moreover, it cannot be said that investigation of this kind has yet been made on such a scale, or with such allowance for the varying circumstances of different populations, as to justify definite conclusion. Such as they are, the results go to show that the proportion of carriers in an ordinary population is about 3 or 4 per 1,000 ; but, for the reasons given, these figures cannot properly be regarded as affording a trustworthy indication of the true proportion of carriers in communities generally.

Dr. Ledingham also discusses the nature of the carrier state and refers to interesting observations that have been made in this connection. He cites statistics which show that, so far as present data go, the chronic carrier condition appears to be much more frequent in adult life than in youth and in the female sex than in the male sex. In the latter connection he considers the association that has been observed between the carrier state and gallstone disease, and the apparently greater liability of women to both these conditions.

A question of practical importance, which Dr. Ledingham treats with considerable detail, is that of the possibility of effecting a cure of the carrier condition. He gives an account of many instances in which curative treatment of the carrier, whether by drugs, vaccines, surgical operation, or in other ways, has been attempted. It has to be confessed that, so far, the results of such treatment have not been encouraging. There would seem to be somewhat more hope of effecting a cure of the urinary carrier than of the intestinal carrier ; as regards the latter, the prospect of success, in Dr. Ledingham's view, is, save perhaps in the early stage of the condition, extremely remote.

The measures that have actually been taken, or that have been suggested as suitable for adoption, with a view to lessening the danger of dissemination of enteric fever by carriers, form the subject of the last chapter of Dr. Ledingham's report. They consist, in the first place, of taking such action as is likely to lead to the detection of carriers in the community, and, in the second place, of endeavouring to secure, on the part of the carrier, the observance of such precautions as are calculated to minimise the risk of his infecting other persons. Foremost among these stands the necessity of the strictest personal cleanliness, especially as regards the hands. Safe disposal of the infectious excreta is also a matter of much importance. There is no legal provision enabling the carrier to be debarred from undertaking what occupation he pleases, notwithstanding the fact that a carrier who is engaged in the milk trade or in the preparation or handling of food may be a public danger. In the absence of power to exclude the carrier from such occupations, endeavour should be made to attain this end by persuasion, and, in the event of failure to persuade, to secure the strict and continued observance of every possible precaution on his part.

THEODORE THOMSON.

CONTENTS.

Page.

Preface by Dr. Theodore Thomson 1

Chap. I.—Introductory 5

Chap. II.—General facts regarding carriers.—Age, Sex, Frequency, &c. ... 9

Chap. III.—Instances of infectivity of carriers.—House and Street cases ... 29

Chap. IV.— Do. do. —continued. — Milk-borne cases ... 42

Chap. V.— Do. do. do. —Institutional cases ... 56

Chap. VI.— Do. do. do. —Army cases 63

Chap. VII.—Pathogenesis of the typhoid-carrying state, with particular reference to the intestinal carrier 69

Chap. VIII.—Physical disabilities of the carrier-state, Auto-infection, &c. 80

Chap. IX.—Treatment of typhoid-carriers, intestinal cases 84

Chap. X.—Urinary carriers. Pathogenesis of the condition and treatment 92

Chap. XI.—Diagnostic methods employed in the search for carriers ... 99

Chap. XII.—Discussion of the general question of carrier-infectivity ... 106

Chap. XIII.—Statistical data regarding the infectivity of carriers ... 113

Chap. XIV.—Immunity-questions in carriers 118

Chap. XV.—Various measures either employed or suggested to diminish the spread of infection by carriers 121

Appendix.—Bibliography 127

CHAPTER I.

INTRODUCTORY.

In the campaign against infectious diseases, no knowledge can be of greater importance to the epidemiologist than that which springs from a correct understanding and appreciation of the natural history of the infective agents, whether bacterial or protozoal. It is only within the last decade that the active co-operation of the epidemiologist with the bacteriologist and parasitologist, has led to a much clearer insight into those factors which influence the rise and spread of epidemic diseases. As evidence of such fruitful co-operation, one has only to cite the progress which has been made in our knowledge of the spread of malaria, sleeping sickness and Malta fever. When we come to those bacillary infections associated with disturbances of the intestinal tract (typhoid fever, paratyphoid fever, dysentery, cholera, &c.), we find that up to quite a recent period, most attention was directed, and rightly so, towards rendering innocuous what were recognised to be at least temporary harbourers, if not permanent hosts, of the bacilli. These hosts were almost invariably of inanimate nature (water, soil, air, food, &c.), but much of the evidence on which was based the belief that such hosts constituted permanent seats of vegetation of the infective agents, has now been proved to be erroneous or of an unconvincing character. It is becoming more and more evident in the case of the diseases referred to, that the main habitat of the infecting agent is to be found in the animal organism itself, where it has found a temporary or prolonged lodgment.

In this memoir I shall be concerned solely with the typhoid bacillus and its recently recognised property of leading a saprophytic existence in an organism which may or may not react to its presence in any obvious fashion.

To Robert Koch (1902) must certainly be given the credit of pointing out clearly the necessity of rendering the typhoid patient innocuous to those in contact with him, by efficient disinfection of the excreta, both during the disease and in convalescence, by careful enquiry into, and supervision of the ambulant and abortive cases, and especially of those anomalous types met with so frequently in children.

At Koch's recommendation bacteriological stations were instituted in the typhoid-ridden districts, notably in south-west Germany, for the purpose of testing in actual practice the validity of his dictum that the chief source of typhoid infection is to be found in man himself. These institutes have proved fruitful not only in helping to check the further spread of epidemics already arisen, but also in explaining the causation of many epidemics the source of which could not be traced to contact with the typhoid patient, or to other commonly recognised agencies of infection. In fact, the discovery of the typhoid-carrier was a natural sequence of the systematic bacteriological examination of the typhoid convalescent. That persons apparently quite healthy could harbour typhoid bacilli after an attack of the fever, emit them continuously or periodically in the excreta and thus act as potential sources of infection, was a new fact in the etiology of enteric fever. It had certainly been demonstrated several years previously that the typhoid bacillus might remain for long periods in the gall-bladder, bone-abscesses and other situations, but the exact significance of such finds from the epidemiological point of view had not been thoroughly realised. Such findings, so far as they concern us here, will be referred to in their proper place (*vide* Chap. VII). The true typhoid-carrier, by which is understood a person who excretes the typhoid bacillus continuously or intermittently in the fæces or urine, will demand the greater part of our attention in this monograph.

As much of our knowledge of the typhoid-carrier subject has emanated and still continues to emanate from the bacteriological stations in south-west Germany it may be well here to refer briefly to their constitution and scope of work.

In thus giving prominence to the efforts of the hygienists and bacteriologists in the south-west Germany campaign, I do not wish it to be implied that the discovery of the carrier-condition was entirely a German product or even that the epidemiological importance of the carrier was first grasped by the Koch school. The intestinal carrier—the most important because the most frequent type—undoubtedly came to light as the result of the German campaign-work; but the chronic urinary carrier—the much rarer, though probably more dangerous type—was known (*vide* Chapter X.) at least two years before Koch's famous address in November, 1902, and in this country Horton-Smith (1900) had already called attention to the etiological importance of the latter in his Goulstonian lectures in 1900. There can be no question however, that it is to the concentrated efforts of the German typhoid-campaigners that we owe the larger part of our material for this new chapter in the etiology of typhoid fever.

In the flat south-western portion of Germany, typhoid fever had become so prevalent, particularly in the Regierungsbezirk Trier, the Bavarian Pfalz, and in Alsace-Lorraine, that a general spread of the disease by the floating industrial population of this district to all parts of Germany began to be dreaded. On Koch's recommendation an experimental typhoid station was planted at Trier in 1903, under the directorship of Frosch. Such excellent service was rendered by this station in elucidating the spread of enteric fever, that it was determined to increase largely the number of such institutes where co-operative epidemiological and bacteriological work could be carried out. Full information with regard to the site, organisation and scope of work of these stations is to be found in a Preface to Volume XXIV. of the Arbeiten aus dem Kaiserlichen Gesundheitsamte, and more shortly in the Festschrift presented by the Imperial Board of Health to the Berlin Hygiene Congress of 1907. Further notes will be found in the individual reports of the stations and in the general statistical reports of Frosch (1907 *b*), Schneider (1907), Kirchner (1907), and Klinger

(1909), which will be further referred to.[*] According to the latter there were in existence and in full swing in the year 1907, eleven such stations with a staff of 35 bacteriologists attached to them.

The institutes were situated at the following places:—

Trier Saarbrücken Saarlouis Neunkirchen	Prussian stations in the district of Trier.
Kaiserslautern Landau	Bavarian stations in the Pfalz district.
Idar	...In the Fürstentum of Birkenfeld.
Strassburg Hagenau	In Lower Alsace.
Metz Diedenhofen	In Lorraine.

The population of this district was about 3,186,000, distributed in 3,240 Gemeinden (communities). The following facts with regard to the constitution of the stations have been extracted practically verbatim from the official Congress Festschrift above referred to (pp. 114, 115, 116).

The typhoid stations are provincial establishments. To ensure uniformity of action and co-operation among them, an "Imperial Commissioner for the typhoid campaign in the south-western portion of the Empire" was appointed in November, 1904, with his headquarters at Saarbrücken. For Bavaria a District Commissioner was entrusted with this duty. In the execution of his office this official would have the assistance of the respective municipal authorities. He has to supervise the general hygienic conditions of his district so far as they may have concern with the spread of enteric (*e.g.*, drinking water, sewage disposal, housing, &c.) and point out deficiencies therein.

For this purpose a medical advisory officer is accredited to his staff.

With regard to the stations, by a special arrangement with the Imperial Administration and that of the confederated States, a definite line of action or "reference" (Dienstanweisung) has been drawn up in which it is expressly stated that their chief task is the bacteriological examination of suspected material from patients with a view to accurate diagnosis. The medical men at these stations are empowered to prosecute their enquiries on the spot in conjunction with the official authorities. In searching out concealed cases, they will have at their disposal the police notifications of strangers in the district, the school absentee lists, dispensary lists, the official death notifications, the lists of employees in manufactories, &c. Further they may derive information from the clergy, the school teachers, and the midwives.

The aim of these enquiries is to search out as early as possible all typhoid cases and carriers, to explain the source of infection in each case, to take active measures against the existing focus of infection, and to prevent the further spread of the disease.

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[*] The organisation of the campaign and the results achieved have been dealt with recently by Lentz in a paper read before the Section of Bacteriology, Brit. Med. Assoc., London, 27th July, 1910.

The directors of the stations have to co-operate with the official health officers, practising physicians and police and parish officials and to accommodate themselves to the directions of the local authorities. They must keep themselves continually informed as to the hygienic conditions of the district and other conditions relative to the occurrence and spread of enteric fever.

Every three months the Institutes make a report to the District Chairman and to the Imperial Commissioner, who presents a return to the Imperial Board of Health.

Special instructions are also given with regard to notification, diagnosis and prevention of the spread of enteric fever. From these I make the following extracts :—

1. Notification must be made not only of undoubted cases of typhoid fever but also of cases suspected to be typhoid fever.

2. In the diagnosis of the disease, the assistance of the director of the bacteriological station as well as of the official health officers should be requisitioned. These should have access to the sick-bed so far as the medical attendant considers advisable in the interests of his patient.

3. Among the measures to prevent the spread of the disease, the removal of the sick and of suspected cases is provided for. Persons excreting typhoid bacilli, but who present no symptoms or only slight symptoms of typhoid fever (so called Typhus-bazillenträger), and convalescents who are still discharging the specific bacilli, should as far as possible be dealt with as sick persons.

Where no legal right exists for this purpose, or where for other reasons (interference with occupation, &c.) such a measure is impracticable, one should by oral and written instructions urge upon these carriers the necessity of disinfecting their excreta and their washing, and convince them that by neglect of these precautions they run the risk, not only of re-infecting themselves, but also of continuously giving rise to fresh cases in their vicinity.

Typhoid patients should be taken to hospital and should be isolated there until two examinations at weekly intervals demonstrate the absence of typhoid bacilli from their excreta.

Where possible, isolation should continue until a third examination, made after another week, has proved negative. Suspected contacts should be kept under observation for three weeks, and, when circumstances demand it, also examined bacteriologically.

In the following chapter I shall deal with the general data regarding carriers, collected from the reports of individual institutes and official campaign statistics in Germany and other countries.

CHAPTER II.

GENERAL FACTS REGARDING CARRIERS.—AGE, SEX, FREQUENCY, &C.

The earlier efforts of the typhoid institutes in south-west Germany contributed an enormous amount of information on the part played in the spread of typhoid fever by contact of the affected person with the healthy, and abundantly confirmed the views promulgated by Koch. An excellent account of the investigations at Trier during the earlier months of the campaign is given by Frosch (1903) and at Saarbrücken by Drigalski (1904). These investigations, together with those of Dönitz (1903) in Berlin, served to illustrate the significance of atypical enteric fever in children, and of the ambulant, abortive, or completely unrecognised types of the disease. Although, however, contact infection from the sick to the healthy was a powerful, if not the most powerful factor in the spread of enteric fever, they found that there was a fairly large quota of recurrent outbreaks of typhoid fever, mostly confined to isolated houses or groups of houses in country districts or streets, where no such contact with an obviously sick person could be traced. This circumstance, together with the knowledge acquired many years before, that the typhoid bacillus might in certain cases remain for long periods in the body in association with bone abscesses, cholelithiasis, &c., led Frosch to put forward the suggestion that possibly in the intestinal tract also the infective germs might lead a saprophytic existence, and by their intermittent discharge into the outer world lead to further infection. Frosch's suggestion was a happy one, and has been entirely confirmed not only by the subsequent investigations in south-west Germany but in all countries where the subject has received attention.

To Drigalski (1903 and 1904) belongs the merit of establishing on a bacteriological basis the hypothesis put forward by Frosch, then Director of the Typhoid Commission, that the B. typhosus might in certain cases lead a prolonged saprophytic existence in the human intestine. When he wrote the account of his experiences at Saarbrücken, this institute had been in existence only ten months. During that time he had been much impressed by the repeated occurrence of enteric fever in certain houses and in towns where the sanitary conditions appeared quite satisfactory. To explain this phenomenon he had to assume the possibility of a prolonged existence of the typhoid bacillus outside the body, but this view he very soon abandoned in consequence of the bacteriological results he obtained in a series of typhoid cases. The stools in 64 cases of typhoid fever were examined bacteriologically at different times during the disease and also during convalescence with the following results :—

Time.	Typhoid bacilli present.		Percentage.
1st.—5th day of the disease...	10 cases.	...	15·6
6th.—10th ,, ,, ...	15 ,,	...	23·4
11th.—20th ,, ,, ...	21 ,,	...	33·0
21st.—27th ,, ,, ...	8 ,,	...	11·5
After 8–10 weeks	7 ,,	...	11·0
After 3 months and later ...	3 ,,	...	4·7

The longest time during which the B. typhosus was demonstrable in the stools was 14 weeks, and in a footnote, referring to a later date, he observes that in this particular case the bacilli were still present at the end of nine months when they formed 50–60 per cent. of the total flora. This case would, therefore, constitute the first recorded chronic carrier traced from convalescence onwards. He was also further successful in discovering a chronic carrier in the person of a Hausfrau who apparently had never suffered from typhoid fever.

Some of these facts which had been detailed a few months previously by Drigalski at a scientific meeting, together with certain parallel observations on convalescent urinary carriers made by Dönitz (1903) in Berlin, had afforded a reasonably strong foundation for the hypothesis formulated shortly afterwards by Frosch.

Before proceeding to detail the systematic bacteriological investigations which have enriched our knowledge of the carrier question, it may be well to refer briefly to the facts which have accumulated regarding the excretion of typhoid bacilli by the typhoid patient during the course of the disease.

In the first place with regard to the faeces, it would now appear that the proportion of instances in which the typhoid bacillus may be detected in the faeces at one or another stage of the disease depends almost entirely on the degree of efficiency of the bacteriological methods in current use. I have already referred to the earlier data of Conradi whose highest percentage (viz., 33 per cent. in a series of 64 cases) was obtained during the third week of the disease, whereas in the first five days only 15·6 per cent. were positive. Much higher values than these have been recorded of late.

Brion and Kayser (1906) examined a series of 144 cases during the febrile period with the following results :—

1st week.	Cases examined	22.	Positive	7	=	32 per cent.
2nd ,,	,, ,,	51.	,,	18	=	35 ,,
3rd ,,	,, ,,	71.	,,	31	=	45 ,,

Bohne (1908) examined 27 cases, and obtained the highest percentage during the fourth week, but his series is too small for statistical purposes.

1st week.	Cases examined	2.	Positive	0	=	0 per cent.
2nd ,,	,, ,,	13.	,,	3	=	23 ,,
3rd ,,	,, ,,	7.	,,	3	=	43 ,,
4th ,,	,, ,,	4.	,,	4	=	100 ,,
5th ,,	,, ,,	1.	,,	1		

By the aid of the more recently introduced media (see Chapter XI.), Gaehtgens and Brückner (1910) have obtained, in a series of 72 cases, the following results which may be considered the best attainable at the present time:—

1st week.	Cases examined	21.	Positive	12	= 57	per cent.
2nd „	„ „	32.	„	17	= 53	„
3rd „	„ „	13.	„	10	= 77	„
4th „	„ „	4.	„	2	= 50	„
5th „	„ „	2.	„	2		

To summarize, in about 75 to 80 per cent. of typhoid cases examined during the third week, typhoid bacilli are demonstrable in the faeces, and it is not unlikely that with further improvements in technique a still higher percentage will be obtained. I have not alluded to the demonstration of typhoid bacilli in the faeces during the incubation stage, but it is important to remember that in not a few instances (Mayer, G. (1903), Conradi (1907), Prigge (1909), &c.), the discharge of the specific micro-organisms at this early period has been proved beyond all doubt.

With regard to the urine, the isolation of typhoid bacilli, if present in this fluid, is, as a rule, a simple matter. Most observers are agreed that in about 25 per cent. of all cases of typhoid fever it is possible to demonstrate the bacilli in the urine. They appear generally in the later stages of the disease, after defervescence (Herbert (1904)), or in late convalescence, but they have also been found frequently at the period of eruption of the rose spots. Further data on this subject will be found in the Chapter on Urinary Carriers.

I now pass to the consideration of Lentz' (1905) results obtained in the course of his examination of typhoid convalescents, at his institute in Idar. Out of 400 convalescents Lentz reported that six continued to excrete the bacillus for a longer period than 10 weeks, dating from the beginning of the disease or from the beginning of a relapse. None of these six cases, however, continued to be infective for a longer period than 13 months, when repeated examination showed that the excreta were typhoid free. There was thus an apparent late cure in the bacteriological sense, but as we shall subsequently show, such "cures" must be regarded with great caution.

Though these six persons ultimately became typhoid free, 12 others did not rid themselves of the bacilli once they reached the chronic stage. The total percentage of carriers in Lentz' series was therefore 4·5 (1·5 per cent. temporary carriers and 3 per cent. chronic carriers).

The fact that these chronic carriers had been traced from the primary infection onwards, led Lentz to investigate all relatives and other persons who had been in association with typhoid patients as well as those persons who in previous years had suffered from typhoid fever. Twenty-two carriers were discovered by this method, 19 of whom were females and three males. The general health of the majority of these carriers was good, but three of the females developed gallstone disease, as was proved by the excretion of small stones in the faeces.

It is not possible to obtain accurate data regarding the previous history of every person who is discovered to be a carrier. As we shall see later, a large proportion of these carriers have not been found to present a history of known typhoid infection. Others again are able to supply data which at least suggest that they may have previously suffered from some form of ambulant or abortive typhoid fever, while others have been traced to the chronic stage right from the primary infection. Different terms have been employed by different authors, in the classification of carriers, but all, in the absence of further knowledge, must be considered somewhat artificial.

Conradi (1907) adopted the classification of Primary, Secondary, and Tertiary carriers. To the first group (Primary) belong those carriers in whom the infection has not led to the occurrence of symptoms of enteric fever. In this group Conradi includes those cases in which the excretion of typhoid bacilli has been found to take place during the incubation stage. The facts relating to this group will be fully discussed later.

To the second group (Secondary carriers) belong those who, having been attacked by enteric fever, continue to pass the bacilli for some time beyond a period of ten weeks from the commencement of the attack.

Out of 400 typhoid convalescents, Conradi got 22 secondary carriers (5·5 per cent. Compare Lentz' figure of 1·5 per cent).

To the third group (Tertiary) belong those who continuously pass the bacilli after the primary attack. Two tertiary carriers out of 400 convalescents were found by Conradi (equals 0·5 per cent. Compare Lentz' figure of 3 per cent.).

This classification of Conradi's is not to be recommended as the terms employed raise no clear idea in the mind as to the conditions they connote.

Sacquepée (1910) has recently suggested a more intelligible classification of carriers, thus :—

Group I. *Porteurs précoces* (precocious carriers), *i.e.*, those who have taken the typhoid bacillus into the system but who as yet do not present any symptoms of infection. This group would consist only of carriers in the incubation stage.

Group II. Persons who have recovered from typhoid or some typhoid-like infection, but who continue to excrete the bacillus whether for a short or for an indefinite period.

Sub-group A. *Porteurs convalescents*, in whom the excretion ceases before the end of the third month.

Sub-group B. *Porteurs chroniques*, in whom the excretion lasts for an indefinite period.

Group III. *Porteurs sains* or *porteurs paradoxaux*, *i.e.*, those who have never had symptoms of typhoid fever, but who may excrete the bacillus over an indefinite period.

It will be seen that the primary carriers of Conradi include both the precocious and the paradoxical carriers of Sacquepée.

There is no doubt that this classification by Sacquepée is a fairly comprehensive one, but a sharp line of demarcation cannot always be drawn between Group I. and Group III. ; and probably many of the so-called paradoxical carriers would be found to fall into the category of the convalescent or chronic carriers if sufficient details as to their previous history were available.

For ordinary descriptive purposes we shall find that the terms " temporary " (or transitory) and " chronic " carriers are sufficiently explicit to denote the two great groups with which we have to deal. According to current German usage, convalescents who excrete the typhoid bacilli for not more than three months after the beginning of the disease are called *Bazillenträger* (or, shortly, transitory carriers), while those who excrete the bacilli for more than three months are called *Dauerträger* (or chronic carriers).

In the course of this survey we shall find that very important differences exist between these two great groups of convalescent carriers.

Having dealt with the figures of Lentz and Conradi, I now proceed to consider further data concerning the frequency of carriers.

Klinger (1906) of the Strassburg station instituted a bacteriological examination of healthy persons living in the neighbourhood of actual typhoid cases. 1,700 such persons were examined, with the result that 15 carriers were found during the period 1st July, 1903, to 31st March, 1905. These carriers were of all ages from 18 months to 60 years, and as to sex, seven were females and eight males. Of these 15 carriers, 11 had no clinical symptoms either before or after finding the bacilli. In nine of these 11 carriers the bacilli were found only on one occasion, but in the remaining two they were found three times in the course of a fortnight. All subsequent examinations were negative. Thus one might provisionally apply the term of " temporary," or as Klinger suggested," acute," to these 11 cases (they were six males and five females). The other four carriers were of the chronic type (two males and two females). One had had enteric thirty years before, a second, 10 years before, while the remaining two were lunatics as regards whose previous illnesses no trustworthy information was available.

Klinger also gives a report of the examination of 482 cases of typhoid during convalescence (at the Strassburg station). Fifty-five or 11·4 per cent. of these excreted typhoid bacilli during convalescence, but not for a longer period than six weeks after defervescence, *i.e.*, temporary carriers. Eight, however, continued to excrete the bacillus beyond this period and thus fell to be reckoned as chronic carriers (= 1·7 per cent.).

In a later communication Klinger (1907) notes that of 604 convalescents examined, he had found 80 temporary carriers (70 or 11·6 per cent. intestinal and 10 or 1·7 per cent. urinary). Six cases only or 1 per cent. became chronic carriers. He states that the temporary urinary carriers were all cured by urotropin.

Another interesting series is reported by Kayser (1907), also of the Strassburg station. This author initiated a late control examination of the typhoid convalescents who had previously been discharged as typhoid-free during the period summer, 1903, to summer, 1905. This late control examination took place after the lapse of at least one year. The potential material for this test was 217 cases, but he was able to recover samples of stools and urine from 101 persons only (= about 46 per cent. of the survivors)

(33 men, 47 women, and 21 children). There had been 248 cases treated, but 29 of these had died. Of the remaining 219, one had died of another disease, and one had committed suicide. The result of this late examination was that three more carriers were discovered (two females and one male). In the previous year he had examined in conjunction with Brion (Brion and Kayser, 1906), the faeces of 200 convalescents and obtained a carrier percentage of 1·5. To show the importance of this late control test, the bacteriological findings in two of Kayser's cases may be quoted :—

Female (40 years)	July, 1904	Primary attack.
	Aug., 1904, 2 exams. ...	Negative.
	Oct. 4, 1905	Negative.
	Dec. 7, 1905	Positive.
	Jan. 18, Feb. 17, Mar. 9, 1906	Negative.
Female (26 years)	Aug., 1904	Primary attack.
	Sept. 23, 1904	Positive.
	Oct. 1, 1904	Negative.
	Dec. 7, 1905	Positive.
	Jan. 18, 1906	Positive.
	May 14, 1906	Negative.

In these two cases, therefore, the routine bacteriological tests made in early convalescence and before discharge, had proved inefficient owing to marked intermittency in excretion. This difficulty is a very serious one and militates greatly against any reasonably practicable system of bacteriological supervision before discharge. By this late control method, Kayser's percentage of convalescent carriers was raised from 1·5 per cent. to 3 per cent. or 5 per cent. I shall have occasion to refer again to the question of enormously prolonged intermittency in convalescent carriers.

A more recent report by Brückner (1910) on a similarly conducted investigation at the Strassburg Institute, shows clearly the necessity of the late control examination of typhoid convalescents. Kayser's material was derived from the town of Strassburg only, while Brückner also included four suburbs of Strassburg and eleven other townships belonging to his campaign district. The potential sources of material were 566 persons who had had enteric fever in previous years. Of these it was found that 45 (*i.e.*, 8 per cent.) had died and 170 (*i.e.*, 30 per cent.) had removed elsewhere or were not discoverable. There were thus left 351 persons, and only 35 of these (*i.e.*, 9·9 per cent.) declined to supply material for examination. This percentage would have been still lower had it not been for one small township in which 21 recovered typhoid cases resided, of whom 12 (*i.e.*, 57 per cent.) refused specimens. Brückner remarks that this low percentage of refusals is an index of the public appreciation of the efforts of the typhoid-stations. Among the 316 persons whose excreta were examined 12 carriers were found (= 3·8 per cent.), viz., nine typhoid carriers and three paratyphoid (B)-carriers. Of these 316 persons 104 were children (under 15 years) and 212 were adults (over 15 years). One carrier belonged to the former group and 11 carriers belonged to the latter group. The proportion of carriers among adults was therefore 5·2 per cent.

I append the following brief facts with regard to the individual typhoid carriers, as they show how important this question of intermittency has become :

1. Frau L. Had typhoid fever in summer, 1906. 19th June, faeces positive, urine negative. 27th July, faeces positive, urine positive. 1st August, faeces negative, urine positive. 13th August, faeces negative, urine positive. Further examinations on 17th, 21st, 27th August, and 17th October, 1906, were negative and Frau L. was registered as "bacteriologically cured." Now, in 1908, typhoid bacilli have been repeatedly found in her faeces, and once in her urine.

2. Herr L. Had typhoid fever in summer, 1906. During the period September, 1906, to April, 1907, he was regarded as a carrier, but as three negative examinations followed on 7th June, 23rd September and 27th October, 1907, he was struck off the list. Now on 11th December, 15th December, 1908, and 2nd January, 23rd January and 20th March, 1909, he is found to be discharging typhoid bacilli.

3. Male (58 years). Had typhoid fever in 1880 and was found to be a carrier in 1906. The discharge was found to be intermittent, but as the result of eight successive negative examinations, he was struck off the list in October, 1906. He is now actively discharging typhoid bacilli.

4. Female (32 years). Had typhoid fever in October, 1906. Discharged as "bacteriologically cured" after three negative examinations. Now her stools are positive (March, 1909).

5. Female (16 years). Had typhoid fever in 1906, and was discharged as typhoid-free. In February, 1909, her stools were found to contain typhoid bacilli. It was of interest that a sister of this case had also had typhoid fever about the same time in 1906, and had been a transitory carrier. The latter, however, is now (March, 1909) negative.

6 and 7. Two brothers (18 and 24 years) had typhoid fever in 1898. Now, in 1909, both are found to be carriers.

8. Frau R. (49 years). Had typhoid fever in 1899. Now found to be a carrier (March, 1909).

9. Male (31 years). Had typhoid fever in 1907 but was discharged as "bacteriologically cured." On 19th November, 1908, his stools contained typhoid bacilli.

In this list there are five males and four females—a relative proportion which is at variance with the usual experience. It has to be noted, however, that two of the males had already been known as carriers but had been struck off the list owing to repeated negative tests. Also in the two brothers (6 and 7) bacilli were found on one occasion only, while in No. 9 one examination only was possible. Further, in the male cases (with exception of 2 and 3), bacilli were as a rule very scarce, while in the female cases, they were being discharged in enormous numbers. Moreover, account has to be taken of the smallness of the series (viz., 9) at Brückner's disposal. Such small series are of little value for statistical purposes.

Perhaps the most thorough bacteriological investigation of typhoid convalescents hitherto reported has been carried out by the Central Research Institute at Kasauli in India, under the direction of Lieut.-Col. Semple and Captain Greig, with the assistance of Lieut.-Col. Thomson, Captains Harvey, White, Hodgson and Assist.-Surg. Paras Ram. (Semple and Greig 1908.)

This enquiry was set on foot in 1906 with the object of elucidating the incidence and spread of typhoid in the Indian cantonments, and one of the main problems attacked was the duration of the typhoid bacillus within the human host. Numerous observations were also made on the viability of the bacillus outside the human body, in urine, fæces, articles of clothing, &c., and the conclusion was arrived at that under natural Indian conditions its saprophytic existence outside the human host was a remarkably short one—a view which practically all modern work on this subject substantiates. In the case of convalescents the observers were not content with the prescribed German routine of two or three final examinations (at intervals of ten days after defervescence) of the excreta before discharge. All their convalescents were examined daily and many of the cases also, throughout the febrile period as well, until the excretion of the bacillus either ceased spontaneously or became chronic. In their report issued in 1908 they note that 86 convalescents had been examined at the Kasauli depôt, with the result that no fewer than ten persons were found to be excreting typhoid bacilli in the urine or fæces for periods longer than six weeks after defervescence. This gives the very high percentage of 11·6 of chronic carriers (taking the three-month limit and over as the test for the "Chronic" category). This is undoubtedly the highest percentage so far recorded and may probably be attributed in great measure to the elaborate routine by which it was scarcely possible to overlook an intermittent carrier. In civil conditions certainly, methods equally thorough would be impossible in practice.

Semple and Greig were working with a more or less homogeneous population, viz., adult males of a definite age-group. In order to determine whether this fact would in some measure account for the high percentage of carriers, I have taken the accumulated statistics of Klinger for the German campaign and determined whether in the age-group 23–45 it would be possible to obtain a percentage of 10 carriers in a small sample of 80 convalescents. The chances of this happening worked out at less than one in a billion, and one must, therefore, conclude that the high percentage they obtained was most probably due to the thorough routine they adopted.

At the close of Semple and Greig's report is a series of tables in which the results of the daily examinations of 18 convalescents are detailed. These tables are worthy of careful study, as they show how readily intermittent carriers may be overlooked by a routine, limited to three or four examinations during the six weeks following defervescence. An extremely interesting feature is the large proportion of cases in which a prolonged excretion of typhoid bacilli by the urine took place in addition to the more common and better known form of excretion by the fæces. As a rule, however, the bacilluria ceased spontaneously after a time (without the aid of urotropin, which in Semple and Greig's experience proved of little or no value as an inhibiting agent), while the fæces remained either continuously or intermittently infective. These tables are too lengthy for reproduction here, but the following summary may be of interest :

Case 1.	Defervescence	8 June,	1907.	Bacilluria ceased 1 Oct.,	1907.
				Faeces positive on 25 Nov.,	"
Case 2.	"	26 June,	"	Bacilluria ceased 30 Aug.,	"
				Faeces always negative.	
Case 3.	"	8 July,	"	Bacilluria ceased 8 Sept.,	"
				Faeces positive 21 Oct.,	"
Case 4.	"	14 May,	"	Bacilluria ceased 24 July,	"
				Faeces always negative.	
Case 5.	"	22 Aug.,	"	Bacilluria ceased 2 Oct.,	"
				Faeces positive 8 Nov.,	"
Case 6.	"	22 May,	"	Bacilluria ceased 2 Sept.,	"
				Faeces positive 10 Sept.,	"
Case 7.	"	17 Aug.,	"	Bacilluria ceased 16 Sept.,	"
				Faeces positive 10 Nov.,	"
Case 8.	"	21 Aug.,	"	Urine positive 12 Nov.,	"
				Faeces positive 12 Oct.,	"
Case 9.	"	10 June,	"	Urine positive, 29 Sept.,	"
				Faeces positive 15 Sept.,	"
Case 10.	"	4 Sept.,	"	Bacilluria ceased 12 Sept.,	"
				Faeces positive 18 Nov.,	"
Case 11.	"	30 June,	"	Bacilluri ceased 17 Sept.,	"
				Faeces positive 28 Sept., (? ceased)	"
Case 12.	"	20 Sept.,	"	Bacilluria (? ceased 6 Oct.),	"
				Faeces positive 15 Nov.,	"

The daily observation of these cases extended over a period of less than six months in each instance. It will be of interest to know from further reports what proportion of these intestinal carriers continued to excrete the typhoid bacillus indefinitely in their faeces, and whether a recrudescence of the bacilluria occurred at a later period.

In addition to this complete examination of convalescents, an examination was also made of nursing orderlies who had been in attendance on typhoid cases. Four of these were examined over a long period after they had finished their course and were about to return to their regiments. None of them ever suffered from enteric fever in a typical form. Two of the men, however, were found to be excreting typhoid bacilli in their faeces. Such cases (probably "transitory" carriers or so-called "acute" carriers of Klinger) are of great importance from the epidemiological point of view, and there is abundant evidence that a proportion of these may pass into the chronic stage.

In a more recent report on army carriers in India by Aldridge (1909) it is noted that of 190 men whose excreta were examined, six were found to be discharging the bacilli more than six months after defervescence, *i.e.*, 3·1 per cent. of the convalescents. These were all invalided to England, and further examinations in this country yielded positive results up to 8, 12, 16 and 18 months.

An extensive enquiry into an outbreak of enteric fever affecting the 71st regiment of infantry at the Saint-Brieuc garrison in Summer 1909, has yielded further evidence showing the enormous importance of the atypical and unrecognised cases. The outbreak has been reported fully in all its aspects, epidemiological, clinical and bacteriological by Billet, Le Bihan, &c. (1910). Briefly, the source of infection was stated to have been in all probability contaminated cider, but the chief interest of the enquiry at least in connexion with the subject of this chapter, centres round the bacteriological findings.

There were treated in hospital 142 cases, *viz.* : 33 very severe, 72 moderately severe, 37 benign, with a total mortality of 13 or 9 per cent.

Blood cultures were made in 74 cases during the first week of the disease with 57 positive results. Widal examinations were done in 43 cases about the end of the third week, with 40 positive results. The faeces were examined in 129 cases at the end of 35 days after entering hospital, with 15 positive results, *i.e.*, 11·6 per cent.

In addition, a careful clinical examination was made of all suspects in the regiment, who showed the slightest symptoms of indisposition, and a surprising proportion of these was found to give either positive Widal tests or typhoid bacilli in the faeces. The clinical histories of these atypical or ambulatory cases are of great interest as showing how insignificant the clinical manifestations of typhoid infection may be. Of 57 such cases examined, 14 only showed a rise of temperature lasting three to five days, seven lasting two days, while the majority (34) showed complete apyrexia. Some were out of sorts for several weeks while others returned to their normal condition after only a few days' indisposition. In eight cases, constipation was the only symptom, while in 15, headache, furred tongue and loss of appetite were the predominant symptoms. Diarrhœa rarely occurred alone, being generally accompanied by swelling of the spleen, and sometimes rose-spots.

Other symptoms complained of in individual cases were stiff neck, angina and "rheumatic" pains. The bacteriological results in these cases were as follows :—

Cultures were made of the blood of 39 suspects with seven positive results, also of 53 men, who lived in rooms where typhoid cases had been most numerous, with one positive result. The stools were examined in 64 atypical cases with 13 positive results, *i.e.*, 20·3 per cent., whereas the percentage of convalescent carriers was only 11·6 per cent. (*see* above). There is no record of repeated examinations having been made in these cases, but the results are sufficient to show the important part which unrecognised cases may play in the spread of enteric fever. As Semple and Greig had found in their investigations that nursing orderlies were particularly liable to act as transitory carriers, so also in this French garrison a large number (11·4 per cent.) of "Infirmiers" showing only ephemeral symptoms of the disease, were discharging typhoid bacilli in their stools. Also the conductor of the ambulance in which the typhoid cases were conveyed to hospital, gave a positive Widal reaction and positive stools, although he appeared to be an absolutely healthy individual.

Tsuzuki (1910) records the results of an enquiry into the question of convalescent carriers in a division of the Japanese Army. The circumstances which gave rise to the investigation will be found detailed in Chapter VI. The bacteriological part of the work appears to have been carried out with great thoroughness, the tests being made practically every day during convalescence. The result was that three out of 51 convalescents in hospital continued to discharge typhoid bacilli beyond the three month limit, *i.e.*, carrier percentage of 5·8.

Further, a large number of examinations was carried out on (1) persons known to have had enteric previously; (2) healthy persons belonging to a company in which a recent case of enteric had occurred; (3) persons occupied in the canteen. In all 5,109 examinations were made of 2,785 persons, with the following results:—

—	Known previous infection.	No known previous infection.	Total.
Number of persons examined ...	316	2,469	2,785
Number of carriers found ...	6	2	8
Percentage of carriers	1·89	0·08	0·28

The particulars with regard to the investigation of these 11 carriers are of interest, and I append the following brief notes:—

CONVALESCENTS OF 1908 WHO BECAME CARRIERS.

A. Fell ill 9th June, 1908. Fever lasted 21 days. Bacilli present in faeces with few intermissions up to 26th December, when he was discharged from service.

B. Fell ill 26th February, 1908. Faeces positive on 19th July, but further examinations up to 3rd October negative, when he was released from isolation.

C. Fell ill 21st September, 1907. Relapse on 13th January, 1908. Urine positive on 13th April, but further examinations up to 23rd June negative. Faeces negative. Released from isolation.

CARRIERS WHO CONTRACTED THEIR PRIMARY INFECTION IN 1907.

1. Fell ill 2nd September, 1907. Faeces positive on 17th April, 1908 and on every subsequent examination up to 17th October, 1908, when he was discharged from duty. The urine was also repeatedly positive up to this time.
2. Fell ill 2nd September, 1907. Urine found to be positive on 17th April, 1908 and on all subsequent occasions up to 17th October, 1908, when he was discharged from active service. The faeces were never positive. Urotropin had little or no effect.
3. Fell ill 17th July, 1907. Faeces positive on 8th June, 1908 and with slight intermissions up to 17th October, 1908, when he was discharged from duty. Urine never positive.
4. Fell ill 30th November, 1907. Faeces and urine positive on 24th April, 1908. Examinations of faeces after 13th May were negative, but the urine continued positive till 23rd October 1908, when he was discharged from duty.
5. Fell ill 14th September, 1907. Faeces positive on 7th May, 1908, but all subsequent examinations up to 17th July were negative. Released from isolation.

6. Fell ill 28th August, 1907. Faeces positive on 19th April, 1908 and on subsequent occasions up to 30th June, 1908. Further samples in July, August, and September were negative. Released from isolation on 3rd October, 1908.

The remaining two carriers had never had definite clinical symptoms and might be called "symptomless" carriers. The one had been somewhat out of sorts in August, 1908 but quite fit for duty. On 2nd September he had given a positive Widal reaction (1 in 50), and examination of the faeces was positive up to 30th November, when he was discharged from service.

The other had absolutely no symptoms. He gave a negative Widal reaction and the faeces were found positive on two occasions only, viz., 11th July and 13th July, 1908. On 11th September he was released from isolation.

In America some valuable statistics have been collated by Rosenau, Lumsden, and Kastle (1909) and by Park (1908). The former investigated the question as to how far chronic carriers were responsible for the spread of fever in the district of Columbia.

Three series of examinations were performed.

1. Persons who had had enteric fever in previous years (6 months—20 years previously), and who had been in association with recent cases during 30 days prior to onset.

   Thirty-two such persons were examined with negative results.
2. In the course of enteric studies in special districts, the faeces of 71 persons who had had typhoid fever 1–10 years previously were examined, but all were negative.
3. In order to determine how many persons in Washington during the typhoid season were carriers, they examined from July 16th to November 8th 1,014 samples of faeces, and 26 samples of urine from 993 persons living in 32 blocks of the city.

   Out of this large material they found only three persons carrying the bacillus who did not present any clinical symptoms of typhoid fever.

The first was a male of 50 years who had never had typhoid fever. His wife had typhoid fever 38 years ago. There had been no typhoid fever in any house where he had resided, and he had enjoyed good health all his life. Some six weeks before, he had been out of sorts with a feeling of discomfort in the region of the liver (? typhoid cholecystitis). The bacilli were found only once and he gave a negative Widal reaction. It is unfortunate that no mention is made of the examination of his wife.

The second was a boy of 14 years. The bacilli were got on one occasion only. No one in the family had had typhoid fever except the boy's mother who had an attack 42 years ago. Here also no mention is made of the examination of the mother.

The third was a boy of nine years. The bacilli were found once only in the urine, while the faeces were negative. None of the family had ever had enteric fever, and these were all examined with negative results.

The percentage found (viz., 0·3 per cent.) accords well with that got by other observers in groups of healthy people associated or not associated with typhoid cases. Thus Minelli (1906) instituted a search for carriers in an institution which had been for years free of typhoid. He chose the town prison of Strassburg containing 250 inmates. The excreta of all these were examined with the result that one carrier was discovered. He had been eight weeks in the prison, and asserted that he had never been ill. His serum gave a high Widal reaction (1 in 1,000)—a fact which strengthened Minelli in his belief that the man may previously have had a slight attack of typhoid. He was 37 years old, and, being a vagabond, he may not unlikely have come in contact with typhoid cases.

Minelli's percentage was, therefore, 0·4.

Park (1908) examined in May and June, 1908, the stools of 52 persons who had had typhoid fever during an epidemic at a lunatic asylum eight months previously. Two were still discharging typhoid bacilli.

He also examined the faeces of 16 persons who had had typhoid fever at another asylum (date not given) and found two of these still positive. They had been well for six months. From this small series therefore he obtained a carrier percentage of six per cent. A large number of convalescents (number not given) were examined on leaving hospital when five per cent. were found to be still excreting typhoid bacilli.

I shall close these data gathered by individual observers with the recent report by Prigge (1909) on the carrier statistics at the Saarbrücken institute.

During the last three years they examined at that institute a total of 10,841 persons and discovered 84 carriers which are classed simply into two groups, (1) Temporary carriers who discharge bacilli up to one year, and (2) Chronic carriers who discharge for a longer period than one year.

It appears to me there are good grounds for extending, as Prigge does, the time limit from three months to one year in the case of temporary carriers, as doubtless, even admitting the difficulty entailed by intermittency, a considerable proportion of convalescent carriers who have passed the three-month limit may cease to discharge spontaneously long before a year has elapsed and therefore do not deserve the appellation "chronic" in the ordinary sense of that term.

In the first group he classes—

(*a*) Persons in whom the bacillus was found before clinical symptoms arose, *i.e.*, Primary carriers (Conradi) or Porteurs précoces (Sacquepée).

Three such carriers were discovered in whom the symptoms did not supervene till 18, 19 and 20 days afterwards. Two of these cases were children and the third was an adult man.

(*b*) Clinically cured persons who continue to discharge bacilli from ten weeks to one year after the attack.

There were 25 of these, viz., 9 men and 16 women.

(*c*) Healthy persons, 24 (16 men and 8 women).

In the second group he classes—

(*a*) Persons who have previously had enteric fever, 27 cases. The proportion of females to males in this small series Prigge does not state, but he notes that in a larger series of 102 cases taken from the whole campaign district, 17 were males and 85 females, thus showing the enormous preponderance of female chronic carriers.

(*b*) Persons who have never had typhoid fever, 8 cases, all women.

The difficulty of obtaining accurate past histories in these cases is, as other observers have found, very considerable, and there is little doubt that some at least of these cases may have had enteric fever in a slight or atypical form.

I come now to consider the carrier statistics compiled for the whole campaign district of South-West Germany from the commencement of the campaign up to the end of the year 1907. These have been recently very fully worked out and commented upon by Klinger (1909), now of the Kaiser-Wilhelms Academie in Berlin. The population of the district (excluding the Bavarian Pfalz) was at 1st December, 1905, about 2,300,000, distributed in 2,532 Gemeinwesen (communities).

Up to the close of 1907, 431 notifications of transitory and chronic typhoid carriers were made (three months being taken as the upper limit for a transitory carrier). These are arranged in the annexed table (reproduced from Klinger's report) according to age and the occurrence or non-occurrence of a definite primary infection.

TABLE I.

Age-Groups.	Chronic Carriers.						Transitory Carriers.					
	Previous Typhoid.			No Previous Typhoid.			Previous Typhoid.			No Previous Typhoid.		
	M.	F.	Total.	M.	F.	Total.	M.	F.	Total.	M.	F.	Total.
0–2 ...	—	—	—	—	—	—	1	3	4	3	2	5
2–5 ...	1	—	1	1	—	1	1	2	3	4	6	10
5–10 ...	1	2	3	—	1	1	4	9	13	11	11	22
10–15 ...	—	1	1	—	2	2	3	2	5	8	5	13
15–20 ...	2	4	6	—	—	—	2	11	13	4	10	14
20–25 ...	4	4	8	—	3	3	9	5	14	2	3	5
25–30 ...	1	13	14	1	3	4	2	6	8	7	5	12
30–35 ...	2	21	23	1	2	3	—	3	3	2	4	6
35–40 ...	3	18	21	—	6	6	2	9	11	6	1	7
40–45 ...	6	21	27	—	3	3	1	6	7	3	2	5

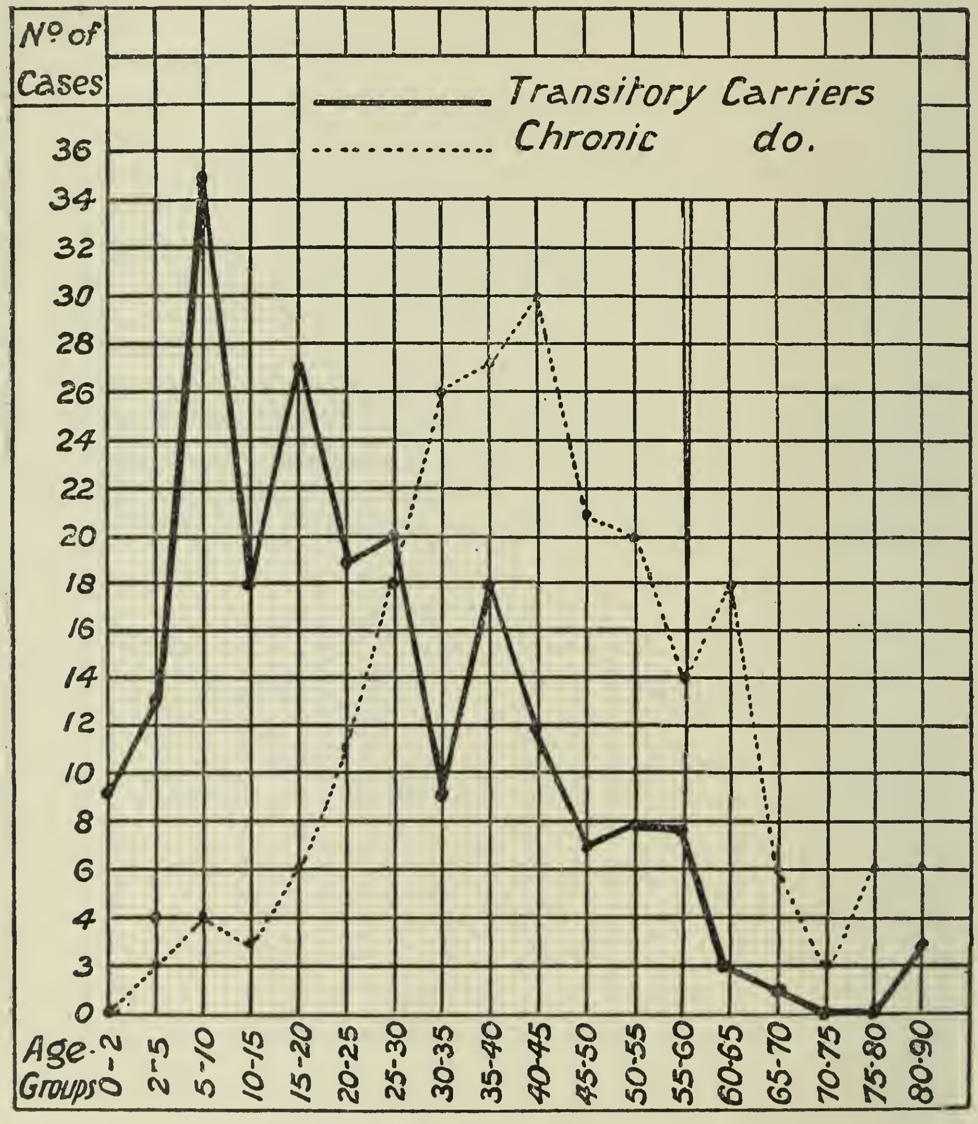
Nº of Cases
Transitory Carriers
Chronic do.
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
3
0
Age Groups
0-2
2-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
50-55
55-60
60-65
65-70
70-75
75-80
80-90

TABLE I.—*continued.*

Age Groups.	Chronic Carriers.						Transitory Carriers.					
	Previous Typhoid.			No previous Typhoid.			Previous Typhoid.			No previous Typhoid.		
	M.	F.	Total.	M.	F.	Total.	M.	F.	Total.	M.	F.	Total.
45–50 ...	1	18	19	—	2	2	—	1	1	2	4	6
50–55 ...	4	12	16	1	3	4	2	1	3	2	3	5
55–60 ...	1	7	8	—	6	6	3	3	6	1	1	2
60–65 ...	2	13	15	1	2	3	—	—	—	1	1	2
65–70 ...	2	3	5	—	1	1	—	—	—	1	—	1
70–75 ...	—	—	—	—	2	2	—	—	—	—	—	—
75–80 ...	2	3	5	—	1	1	—	—	—	—	—	—
80–85 ...	1	1	2	—	—	—	—	—	—	—	1	1
? ...	—	2	2	—	2	2	1	—	1	1	2	3
Totals ...	33	143	176	5	39	44	31	61	92	58	61	119

There were thus 220 chronic carriers and 211 transitory carriers. Of the former the great majority (80 per cent.) gave evidence of a primary infection, while in only 43·6 per cent. of the transitory carriers was this evidence forthcoming.

The enormous preponderance of female chronic carriers over male is strikingly brought out (viz., 182 to 38, or nearly 5 to 1), whereas among the transitory carriers the proportions are very nearly equal (female to male, 1·4 to 1).

Gallstone troubles were definitely diagnosed in 30 chronic carriers, *i.e.*, 13·6 per cent. This figure corresponds closely to the percentage of gallstone cases (of all origins) in which diagnostic symptoms are present.

Perhaps the most important and striking feature brought out by the Table is the different distribution of the transitory from that of the chronic carriers according to age groups. Whereas among the transitory carriers young persons form the great majority, among the chronic carriers persons in middle age or advanced life predominate markedly.

This is shown more clearly in the annexed curve in which the maximum point for the transitory carrier is reached at the 5–10 age limit and that for the chronic carrier at the 40–45 age limit. It is important to note, however, that the curves indicate only the actual incidence on the several age-groups and not the proportional incidence.

With regard to the period during which these carriers have been under bacteriological control since they passed the three-month limit, 30 have been examined up to six months and the others from one year to two, three, four and four-and-a-half years.

Included in the 220 chronic carriers are some 30 cases in which the primary infection took place 4–30 years previously.

A spontaneous cessation in the excretion of the typhoid bacillus occurred in 42 of these chronic carriers, sometimes indeed after prolonged positive periods, but as Klinger remarks, such bacteriological cures must be regarded with considerable scepticism in view of the prolonged intermittent periods which have been observed and the not unlikely possibility of the transmission of a false sample.

During 1906 and 1907, 38 typhoid convalescents or 1 per cent. of the total number of typhoid cases (3,867) became chronic carriers. The percentage is thus much smaller than that obtained by individual observers.

Further facts with regard to the campaign particularly during the earlier years 1904, 1905 and 1906 are given by Frosch (1908). There was a total of 6,708 enteric cases in that period (beginning of 1904–end of 1906), and of these 310 became carriers; 166 excreted the bacillus for three months or longer, *i.e.*, 2·47 per cent. (= Dauerträger) and 144 excreted the bacillus for a period under three months, *i.e.*, 2·15 per cent. of transitory carriers (= Bazillenträger).

In Frosch's series females again formed 82 per cent. of the chronic carriers and 60 per cent. of the transitory carriers, while children (up to 15 years) formed only 4 per cent. of the chronic carriers, but 35 per cent. of the transitory carriers.

An interesting point with regard to the occupations of the female typhoid cases, is brought out from Frosch's figures. For the year 1906, the total number of typhoid cases in the district was 2,080 (938 males, 454 females, and 688 children up to 15 years). Of these 454 females no fewer than 314 were "Hausfrauen," while 56 were domestic servants. Thus 370 or 81 per cent. of the female cases were engaged in housework, and from these the great majority of the chronic carriers and more than half of the transitory carriers were recruited.

I shall deal with the significance of this fact when I come to consider the pathogenesis of the carrier state (*vide* Chapter VII).

The data for the Bavarian Pfalz have been compiled by G. Mayer (1910).

In July, 1903, Bavaria decided to join in the campaign that had been begun in the neighbouring districts of south-west Germany and erected two institutes at Landau and Kaisersläutern respectively. G. Mayer directed the latter up to 1st February, 1908. The population of his district was about 400,000 and the number of typhoid cases was about 930 during the period (1904–1907).

Typhoid carriers to the number of 151 were found, viz., temporary (under three months) 73, chronic (over three months) 78. Of the temporary carriers 54·9 per cent. were women and 57·4 per cent.

children, while of the chronic carriers 65·5 per cent. were women and 25·2 per cent. children (up to 15 years).

Nine of the chronic carriers (or roughly 10 per cent.) suffered from gallstone trouble, while three had severe "bilious" relapses after three, six and ten years respectively.

According to the carriers' own statements, 16 of them had never been ill, 10 had had enteric fever four–nine years previously, and two of them had had enteric fever 20–30 years previously.

"Bacteriological" cures resulted in 62 chronic carriers, four left the Pfalz district and one (a servant girl) drowned herself.

The great majority of the carriers dealt with in this chapter are adult males and females of the chronic type. The records show that children only very rarely become chronic carriers, but their importance as temporary carriers has been repeatedly proved since bacteriological methods have shown how insignificant and how liable to be overlooked a typhoid infection may be in young children.

I do not propose to give actual instances in illustration of the spread of infection by transitory carriers in the persons of young children, but I cannot refrain from quoting one striking case in which a series of typhoid cases was considered to be due to a suckling male child of 18 months. This case has quite recently been reported by Rommeler (1910) of the Neunkirchen Institute. The facts are as follows:—

On 29th November, 1909, a woman who was suckling a child of 18 months developed vague febrile symptoms. Eight days later she was removed to hospital where she passed through a typical attack of enteric fever. Up to the time of her removal to hospital she continued to nurse the child. The latter was then handed over to a female neighbour to be nursed. On the first day of her custody (viz., 8th December, 1909) she noticed that the child had slight diarrhœa, but which she not unnaturally attributed to the necessary weaning.

On 25th December the *foster-mother* developed typhoid fever. On 4th January, 1910, her *six-year old daughter*; on 6th January her *three-year old niece*, who came every day to play in the house; on 18th January her own *suckling child*, and on the same date her *fifteen-year old daughter* fell ill with the same disease. As almost the whole family was attacked, a relative (female of 20 years with some experience in nursing) was called in to take care of the sick. She also took ill on 2nd February and died in six days.

There appeared to be little doubt that the series of cases arose from the suckling child temporarily adopted by the family, but the suspicion was raised to a certainty by the result of the bacteriological examination carried out. On 19th January, 1910, the serum of the child gave a Widal reaction of 1 in 100. Also on two occasions, 21st and 25th January, typhoid bacilli were isolated from the child's faeces, but nine subsequent examinations were negative.

The importance of the atypical and ambulant cases in children is further exemplified in a recent paper by Brückner (1910–*b*).

I close this chapter with some data relative to the so-called "Primary" or "Precocious" carriers in whom typhoid bacilli have been demonstrated in the excreta some time before the outbreak of symptoms. G. Mayer (1910) in a recent paper states that in 1903 he was the first to demonstrate the presence of typhoid bacilli in the stools during the incubation stage. The case was a boy of six years whose stools contained typhoid bacilli eight days before symptoms arose. Although he was immediately isolated, both his brothers contracted the fever. In 1905 and 1907 the same author was able to record two further cases of this nature.

Conradi (1907*b*) gives notes of two cases in which the bacilli were found in the faeces during the incubation stage.

Case I.—Typhoid fever broke out (9th to 18th April, 1906) in a family of eight members, the persons attacked being the mother and three children. On 20th April the cases were brought to hospital, having been nursed up to that date by the grandmother (61 years of age). Typhoid bacilli were isolated from the grandmother's faeces on 2nd May, 1906, and eight days later she also developed typhoid fever.

Case II.—On 27th December, 1903, a man 30 years of age fell ill with typhoid fever and was nursed by his wife till 10th January, 1904, when he was removed to hospital. On 25th January, 1904, typhoid bacilli were found in the healthy woman's faeces and eleven days later she also developed typhoid fever.

The importance of these cases and the infections arising from them (so-called early contacts) have been for some years strongly insisted upon by Conradi who was also able to demonstrate the presence of typhoid bacilli in the blood during the incubation stage. In the beginning of March, 1907, a girl contracted enteric fever. On the 24th March Conradi took a sample of blood from her brother who was at the time quite well and isolated the B. typhosus therefrom. Four days later this boy also fell ill with typhoid fever of a severe type.

I cannot enter in detail into the statistics which have been compiled, notably by Conradi (1907*c*) and Klinger (1909) on this "early contact" question, but I extract from their works the following tables which, it is claimed, show the relatively high infectivity of typhoid patients during the incubation stage and the earlier weeks of the disease.

The material at Conradi's disposal for the investigation of this question was derived from the data relative to certain groups of cases carefully selected from the total of 600 cases investigated during a period of 2½ years, at Saarbrücken. In calculating the date on which the primary case infected the secondary, he took the interval between the beginning of the primary case and the beginning of the secondary and subtracted therefrom 10 days.

In Table I. are recorded the data relative to 38 contact infections (secondary cases) which took place in various families in spite of the fact that the respective primary cases had been removed to hospital. As a rule 12 days elapsed between the beginning of the disease and the removal to hospital. On the day after removal the house was thoroughly disinfected. Conradi considers that the table shows that the great majority of the secondary cases must have received their infection from the primary cases, while the latter were still in the early days of their illness.

In Table II. are given the data with regard to a series of secondary cases which arose in families in spite of disinfection and isolation of the primary case in the house. Satisfactory isolation was admittedly difficult in many cases, so that there may have been some opportunity of infection after the 12th day, when disinfection was, as a rule, performed.

In Table III. are given the data relative to secondary cases due to primary cases not treated by a physician.

TABLE I. (CONRADI, 1907*c*).

Data relative to 38 contact infections which took place in various families in spite of the fact that the first person attacked was removed to hospital.

Infected by	the primary case	during the	1st	week of	the disease,	25	secondary cases.
"	"	"	2nd	"	"	7	"
"	"	"	3rd	"	"	4	"
"	"	"	4th	"	"	1	"
"	"	"	5th	"	"	1	"

TABLE II. (CONRADI, 1907*c*).

Data relative to 28 contact infections which took place in families in spite of disinfection and isolation of the primary patient in the house.

Infected by	the primary case	during the	1st	week of	the disease,	14	secondary cases.
"	"	"	2nd	"	"	7	"
"	"	"	3rd	"	"	2	"
"	"	"	4th	"	"	2	"
"	"	"	5th	"	"	1	"
"	"	"	6th	"	"	1	"
"	"	"	7th	"	"	1	"

TABLE III. (Conradi, 1907c).

Data relative to 19 contact infections due to typhoid cases not treated by a physician.

Infected by	the primary case	during	1st	week of	the disease,	10	secondary cases
"	"	"	2nd	"	"	2	"
"	"	"	3rd	"	"	4	"
"	"	"	4th	"	"	2	"
"	"	"	5th	"	"	1	"

These 85 contact infections may be thus summarised, 10 days being taken as the most probable incubation period.

Infected by	the primary case	in the	1st	week of	the disease,	49	secondary cases.
"	"	"	2nd	"	"	16	"
"	"	"	3rd	"	"	10	"
"	"	"	4th	"	"	5	"
"	"	"	5th	"	"	3	"
"	"	"	6th	"	"	1	"
"	"	"	7th	"	"	1	"

It will be seen that 58 per cent. of the observed contact infections took place during the first week of the primary infection, and in view of the facts that have been ascertained with regard to the infectivity of the incubation stage, Conradi considered it probable that a considerable proportion of these may have become infected during the incubation stage of the primary case.

The table compiled by Klinger (1909) from the data relative to the two years 1906 and 1907 of the typhoid-campaign in south-west Germany, affords a striking illustration of the importance of the primary carrier, in the spread of enteric fever.

The material employed was a series of 812 contact infections, two weeks being taken as the average incubation period. In 60 cases Klinger believed that he had ascertained the incubation period with tolerable certainty ; he found it to vary from 5 to 45 days, with an average of 16 days. Also in the course of a water-borne epidemic he had found that 98 per cent. of a series of 83 cases regarded as having been infected at the same time developed clinical symptoms 11 to 18 days later.

(Klinger, 1909).

Incubation Period.		Course of the Disease.			
1st week.	2nd week.	1st week.	2nd week.	3rd week.	4th week.
(1906) (1907) 18 + 15	84 + 66	104 + 83	87 + 71	58 + 58	16 + 43
33 contact cases.	150 contact cases.	187 contact cases.	158 contact cases.	116 contact cases.	59 contact cases.

Course of the Disease.					
5th week.	6th week.	7th week.	8th week.	9th week.	10th week.
13 + 21	7 + 15	9 + 5	9 + 7	10 + 5	5 + 3
34 contact cases.	22 contact cases.	14 contact cases.	16 contact cases.	15 contact cases.	8 contact cases.

Klinger admits it would be a mistake to place too much reliance on the accuracy of the figures in view of the variations in the incubation period ; nevertheless he takes the view that taken as a whole the figures show that a preponderant number of contact infections take place during the early period of the typhoid infection, or, in other words, that a typhoid patient is most dangerous during the incubation period, and the first and second weeks of the disease, *i.e.*, during the period when little or no precautions can be taken. In the later stages of the disease the gradual cessation in the excretion of the specific bacteria and the precautions taken as to disinfection, &c., serve to keep the contact infections at a relatively low figure.

CHAPTER III.

INSTANCES OF INFECTIVITY OF CARRIERS.

House and Street Cases.

The influence of carriers in maintaining the endemicity of typhoid fever in individual houses and streets, or groups of houses in isolated country districts, has been abundantly attested by the work of recent years, and in no other sphere has the search for carriers been more fruitful. Indeed, so many instances of the dangers arising from carriers in this connexion have accumulated that it will be possible to refer in detail only to the most striking and illustrative. The part played by carriers in the dissemination of enteric fever through the agency of milk or of water and their relationship to the occurrence of the fever in army-units and in institutions will be discussed in following chapters.

I shall commence with the now well-known case of the Strassburg baker's wife, reported four years ago by Kayser (1906*a*).

On 20th May, 1904, an apprentice, lodging with the master baker's wife, a woman 40 years of age, contracted typhoid fever and died during the third week of the disease. The baker's wife was known to have had typhoid fever ten years previously. In recent years it had been observed that practically every fresh apprentice became ill shortly after his entering on residence with her, with symptoms pointing to stomach and bowel disorder. These symptoms were attributed by the baker's wife to "too good diet." On 26th May, 1904, this woman was discovered to be a carrier, and further examination of her stools on 1st June, 3rd June, 22nd June, and 5th July proved positive. From the 19th July onwards, however, weekly examinations of her stools gave negative results; but there was a strong conviction that the samples received from her were not her own.

On 8th August her tenant, a man 29 years of age, who used the same water-closet as the baker's wife took ill and died in the third week of the disease. Under police pressure the woman was now brought to the clinic and her stools were found to contain enormous numbers of typhoid bacilli.

In a later communication Kayser (1907) attributes another case of typhoid fever to infection from the baker's wife.

Thus three cases occurred in the household of this woman.

Second case. Kayser (1906*a*).—A female 40 years of age was discovered to be a carrier in November, 1904, and her stools and urine were examined every three weeks thereafter for about a year. Thirty years previously she had passed through an attack of typhoid fever, and during the past five years she had suffered from severe gallstone colic.

On 20th May, 1904, her son-in-law (27 years) who lived in the same house, fell ill with typhoid fever, as also, on 17th November, did her lodger, who succumbed to the disease. These two persons had used the same water-closet and the same dishes, &c., as this woman. Two other persons, who did not belong to the household, but who came in contact with the woman in a business capacity, also contracted typhoid fever. After the 20th November, 1904, when she was discovered to be a carrier and regulations as to disinfection, &c., were in consequence imposed upon her, no further cases occurred in her vicinity. No record could be obtained of the occurrence of cases in this household prior to 1904.

A number of interesting cases of household infections traceable to carriers are given by Mayer (1908) in his report on the typhoid investigations in the Bavarian Pfalz during 1903–6.

Case 1.—In August, 1906, a girl (13 years) in the hamlet of "V" was notified as a case of typhoid fever. One death from typhoid fever had occurred in this hamlet on the 14th April, but no connexion could be traced between this and the August case. It was ascertained, however, that the grandmother of the girl had had typhoid fever 49 years previously, her grandfather 20 years previously, an aunt 28 years previously, her mother 23 years previously, and a brother 9 years previously. In the course of an examination of these persons, the mother (41 years) was found to be a carrier.

Case 2.—A young married woman was notified in April, 1904, as a case of typhoid fever, and the medical officer stated that her eighteen month's old child had suffered from angina and diarrhœa about 14 days before the commencement of her mother's illness. Where the woman lived there had been only one case of typhoid fever in recent years, but no connexion could be traced between this case and the sick family. The case would have remained puzzling without a knowledge of other cases which had occurred in earlier years in this place. On consulting the registers Mayer found a patient who had had typhoid fever in 1900, and whose name was the same as that of the sick woman. Enquiry established the fact that this man was the husband of the sick woman and the father of the child, and further examination led to the discovery that he was a carrier.

Case 3.—Typhoid fever had been endemic on a large estate in "L" since 1896. Soil contamination had been blamed, but in spite of improved drainage cases continued to crop up. Repeated disinfection of the rooms in which typhoid cases had occurred also failed to arrest the spread. Cases had arisen in the following order:—

1896. The manager himself, and, later in the same year, a girl employed at the farm.
1897. The housekeeper.
1899. A girl, in middle of July, employed on the farm.
1900. Ditto ditto ditto.
1903. Ditto ditto ditto.
1897–8. Three persons in a neighbouring house, where relations of the manager resided.
1900. A labourer living in a house exactly opposite the farm.
1901. A farm labourer and his master, who died.
1902. Two other farm labourers, one of whom died; also a girl in a neigbouring house.

In June, 1906, four persons living in three houses not far from the farm took typhoid fever. Their water supply—a rather unsatisfactory pump-well—was the same as that of the farm and there was a suspicion that it was being polluted from a water-closet employed by the manager of the farm, and, in fact, 14 days before the outbreak of the new cases the manager had been known to use this closet. His faeces were now examined and he was discovered to be a carrier. It was practically certain that for years he had been spreading typhoid fever in his vicinity.

Rosenthal (1906) in Göttingen gives an interesting description of repeated cases in one household attributable to a carrier.

During the autumn and winter of 1905–6 there had been four cases of typhoid fever in a single household and the faeces of 13 inhabitants of this house were examined, with the result that one female carrier (42 years) was discovered. She had not been ill for years but there was some evidence of her having passed through an attack of typhoid fever in 1878. In that year she had had a severe febrile attack and at the same time her son had a severe intestinal trouble. During that year also an epidemic of typhoid fever had broken out in her street. On enquiry it was found that from 1897 to 1906 13 cases of typhoid fever were in all likelihood attributable to personal intercourse with this woman. Six of these persons were lodgers who got breakfast and supper from her; three were children who lived in the house; one was a female neighbour, who often visited her; and three were persons in neighbouring houses who got their vegetables from her. This woman and her husband sold vegetables and fruit and attended the markets three times a week. She may therefore have infected many more people. Rosenthal also notes that the excreta from the house were used to dung the vegetables. Necessary precautions were now enforced with regard to disinfection of excreta, but there was no power to prevent the woman plying her trade.

Mention may now be made of a well-known case reported by Soper (1907) of New York, in which a female carrier in the person of a cook was found to have been responsible for the occurrence of no fewer than 26 cases of typhoid fever in seven different families.

The final outbreak attributable to this cook occurred in the first two months of 1907 while she was in service in a New York family, the victims being a chambermaid and a daughter of the house, who died (the only fatal case in the series). The cook had been two months in this family before the cases arose. On March 19th, 1907, this woman, after a severe struggle in which police aid had to be requisitioned, was conveyed to the detention hospital, under charge of Drs. Wilson and Park, and there, her faeces were bacteriologically examined with a positive result. Much difficulty was experienced in tracing out the past history of this cook and possibly the achievements now to be mentioned have no claim to be exhaustive. Considerable gaps in her history could not be filled up in spite of careful enquiry.

The various outbreaks occurred as follows:—

September, 1900. Mamaroneck. During a summer residence in this place a visitor to the family developed typhoid fever. It is remarkable that the cook had been three years in this family without having given rise so far as is known to any case of the disease.

December, 1901. New York. One month after the cook's arrival in this family a laundress in their employment developed typhoid fever.

Summer, 1902. Dark Harbour. Seven members of the family and two servants developed typhoid fever. All the servants except the cook had been employed in this family for one month or more in New York before leaving for the summer residence in Dark Harbour. The cook had been engaged specially for the summer, and had joined the family three weeks before it left for Dark Harbour.

Summer, 1904. Sands Point. Four servants developed typhoid fever. The cook had been nine months in the service of the family without any untoward occurrence. The cases arose when the family removed to Sands Point, and the servants were practically new hands. (Laundress, gardener, butler's wife, and butler's wife's sister.)

Summer, 1906. Oyster Bay. Six cases occurred in one household.

September to October, 1906. Tuxedo. One laundress took ill 14 days after the arrival of the cook.

January to February, 1907. Final outbreak at New York (already quoted).

In a further report on this case Park (1908) mentions that the cook had been kept in detention since she was discovered to be a carrier, and that various intestinal antiseptics had been employed with a view to cure, but without success. Her urine was always negative.

Niven (Medical Officer of Health, Manchester) (1908), reported the discovery of a carrier in the person of a woman who kept lodgers and did her own housework. The cases ascribed to her occurred as follows :

September 24th, 1908. Male lodger (36 years) developed enteric fever four days after leaving the house.

September 7th, 1907. Male lodger (19 years) developed typhoid fever.

September 4th, 1906. Male lodger (28 years) took typhoid fever and died.

May 2nd, 1906. Male lodger (28 years) took typhoid fever and died.

The following three cases were also traced to two other houses at which Mrs. W. had taken in lodgers :—

May 20th, 1905. Lodger (19 years). This case, however, may have been infected outside Manchester.

April 8th, 1905. Lodger (25 years) developed typhoid fever.

October 4th, 1898. Lodger (23 years) took typhoid fever and died.

In none of these cases was there a history of contact with actual typhoid patients, nor could shell-fish consumption be incriminated. The history of Mrs. W. was briefly as follows :

> Up to her 54th year she had been quite healthy. In this year (1893) she had an attack of influenza and pneumonia. The illness had lasted five weeks, and she had been kept on fluid food. Since this illness she suffered periodically from pain in the abdomen and vomiting. In 1898 the attacks were very numerous and occurred at intervals of three to seven days. This year was marked by the first appearance of typhoid fever among her lodgers.
>
> When the woman was examined in November, 1908, no abnormal physical signs could be made out, the liver was not enlarged and there was no palpable enlargement of the gall bladder. There was no evidence of gall stones, and so far as her own medical attendant knew, she had never been jaundiced.
>
> Examination of her excreta by Prof. Delépine on November 4th, 1908, revealed typhoid bacilli both in faeces and urine. On December 3rd, however, the faeces only contained the bacilli. In November, 1907, her blood had given a negative Widal reaction, but on November, 1908, a positive reaction was obtained.

An account of this case has also recently been given by Hutchinson (1910).

Gregg (1908) (America) reported that seven cases of enteric fever had occurred among the inmates of a small boarding house in a farming town between August, 1905, and March, 1908. The mistress, who prepared all the food herself, had had typhoid fever so long ago as 1856. The faeces of this woman were repeatedly positive, but the blood and urine were negative. She enjoyed excellent health. No other cases occurred in the neighbourhood, and there was no other discoverable source of infection.

Another house-endemic persisting over a long term of years and attributable to a female carrier, has been reported by Huggenberg (1908). Thirteen cases in all occurred in one family during the period 1877–1908, viz. :—

Mother : May, 1877.
Father : July, 1886.
Son : September, 1892.
Female servant : February, 1899.
Do. August, 1899.
Do. September, 1900.
Do. August, 1902.
Daughter-in-law : September, 1902 (had been living for some weeks in the family).
Female servant : February, 1903.
Do. October, 1903.
Do. December, 1904. (This servant was living in the same house but with another family.)
Do. June, 1905.
Do. February, 1908.

Huggenberg made a bacteriological examination of the excreta of all the members of the family and was able to demonstrate typhoid bacilli in those of the mother, who had had her primary attack of enteric fever 31 years before. Her serum also gave a well-marked Widal reaction.

A few new experiences have recently been reported by Kayser (1909) in the course of the investigations at Strassburg.

Case 1.—House case with evidence pointing to the mode of transference of the infective agent from the carrier to the new host.

Frau H. (31 years) had had typhoid fever in her youth. Until August, 1905, she had lived with her three children, aged 4, 7, and 10 years, in a sanitary dwelling, and there had been no cases of the fever. During this month the family removed to a new suburban building in which the water-closet arrangements were not satisfactory, and the well was insufficiently protected from risk of contamination by the excreta of the household. This well formed the household water supply and was not used by other households. The children drank of this well water. On 19th August the seven-year-old daughter fell ill with typhoid fever, as also did the 10-year-old son, who died. It was then discovered that the mother, who had had typhoid fever 14 years before, was a chronic carrier. Nothing had happened while the sanitary arrangements of the house were above suspicion.

Case 2.—Where the medium of transference was in all probability food stuffs.

Frau B. went to a neighbouring town to attend Frau J. in childbirth. Some of the latter's family had had typhoid fever three months before, but all of these during convalescence had been declared typhoid-free. On her return home from attendance on Frau J., Frau B. took with her some fruit and pastry of which she and her husband partook. The husband shortly afterwards developed typhoid fever, and Frau B. was for some days out of sorts, but there was no reason to suspect that her illness was typhoid fever. The household of J. was examined, and Frau J., who was quite well, was discovered to be a carrier.

Herr B. after his attack also became a chronic carrier and received instructions with regard to the disinfection of his excreta. After the middle of February, 1907, he neglected to take the prescribed precautions. In March, 1907, Frau B. had an accident to her abdomen followed by what was thought to be a traumatic perityphlitis, but which proved to be typhoid fever. In Kayser's view the trauma was probably a factor which predisposed to the contraction of the fever (*vide* Chap. XII.).

Case 3.—This case is also interesting as showing how important it is to see that the disinfection of excreta is properly carried out by persons who are found to be carriers.

A shoemaker's wife, Frau E. (50 years), was discovered to be a chronic carrier in September, 1905. In July, 1905, a daughter, and in August–September an adult, son of Frau E., had contracted typhoid fever. The woman objected to the disinfection of her stools and resented interference with her domestic arrangements. Near her house lived a young married daughter of hers who frequently visited her along with her child. The latter took ill in Septemder, 1905, and died, and the daughter herself took ill and died in June, 1906.

Brückner (1910) records another instance of a female carrier who refused to carry out her instructions. For some years she had been under observation as a carrier, but cases attributable to her continued to crop up from year to year, and in 1909 had reached the respectable total of 13, including one fatal case.

This woman decidedly refuses to carry out her instructions and will, therefore, remain a standing danger to the community.

An interesting account of endemic typhoid fever in a country district associated with a carrier case is given by Baumann (1908) of the Metz bacteriological station.

At the end of August, 1906, the station at Metz received notification from the station at Hagenau that a male typhoid patient, Michael M., in the hospital at "S," had probably contracted infection at a farm "G" in the Metz sphere of operations. It was ascertained that Michael M. was engaged only for a short time in the summer at "G," in the capacity of shepherd; that at dinner time only he visited the farm, and slept at night in the fields. At the farm there had been of late no cases or suspected cases of typhoid fever, but at a farm "R," situated half-a-kilometre from "G" a girl Amalie E. daughter of Leonard E., the tenant of the farms "G" and "R," had been suffering for some time from a throat affection. The shepherd had not visited this farm "R." Amalie E. (25 years) was found to have had an illness in "R" and was now recovering. From the symptoms alleged, it was impossible to exclude a diagnosis of typhoid fever. Specimens of her blood and stools had been sent by her physician to the station at Metz, but in spite of the negative report he believed the case to be one of typhoid fever. No notification was made. Baumann accordingly examined blood samples from Amalie E. and also from her sister Fanny E. (31 years), who cooked for the farm "G" where the shepherd had his meals. This girl Fanny affirmed that she had never suffered from typhoid fever or any similar complaint. Five years ago, however, she had been troubled for some time with constipation. In both cases the Widal test was positive. It was therefore tolerably certain that Amalie E., whose first Widal was negative and second positive, had passed through an attack of typhoid. Repeated examinations of the stools of both girls were negative. A sample from Josephine E., who also worked in the kitchen at "G," was negative.

On 28th August a blood sample was received from a day labourer, Magdalena E., living in the same parish, "J," who had been ill in June but had only now called in a physician. This specimen was positive, but her stools and urine were repeatedly negative. During the summer this woman was engaged at the farm belonging to Leonard E., where she also got her midday meal. In this case also it seemed probable that the illness had been typhoid fever.

In the village "J" itself there had been no typhoid cases for several years.

On 20th September, 1906, a day labourer, Anton S. (32 years), belonging to "J," and who also went to work at the farm "G," contracted typhoid fever. His Widal was positive but stools and urine were negative. (This man later infected his two year old daughter Octavia S. who contracted typhoid fever on 26th November, and had a positive Widal and typhoid bacilli in her stools).

Inquiry was now instituted at the farms "G" and "R" (separated by 3 kilometres). At "G" two new cases were found which had not been notified, viz., a son Albert E. (23 years) who took ill on 23rd September and a daughter Louise E. (27 years) who had been ill since 5th October (Widal positive).

A sample of blood was also taken at the same time from the father Leonard E., a very healthy person. It was negative. However, both from him and the other members of the family stool and urine samples were taken. In Leonard E.'s stool the bacillus typhosus was found on 23rd October, but as his stool arrived on the same day as those of the sick members of the family, a suspicion arose that the samples had got mixed. Repeated examination, however, of the stools of Leonard E. proved positive, and there was no doubt that he was a chronic carrier. For several years this man had suffered from gallstone colic.

Further enquiry showed that in the autumn of 1905 in a neighbouring village "W," three persons had been ill with typhoid fever, two of whom worked at the farm "G." The third was the daughter of one of these labourers. In all these cases the Widal test had been positive, one of them had the B. typhosus in the urine and in another both stools and urine contained the bacilli.

Alfons M., brother of Michael M., went as shepherd to the farm "G" in his sick brother's place. He also took typhoid fever (Widal positive. Stools positive). On 16th October three new cases were notified, viz., two members of the family E., living at the farm "G," and a case in the village "M," situated about 5 kilometres from "G." This latter was a five-year-old boy who had been for some time on a visit to his grandfather Leonard E. at "G." It was further ascertained that a brother of this boy (7 years) had had typhoid fever in July and died on 1st August. He also had previously been on a visit to his grandfather Leonard E. The two cases at the farm "G" were a son, Emil E. (19 years) and the servant girl Josephine E. (16 years).

No further cases occurred in the neighbourhood for some months, but on 19th March, 1907, another case in "J" was notified. This was a day labourer (stool and urine positive). On 25th March two further cases were notified from the neighbouring village of "L." These were two farm labourers (23 years and 25 years). Another case was found to be convalescing but gave a positive Widal.

All the four cases just mentioned had been employed intermittently at the farm "G" as day labourers.

Further cases had not occurred in this locality up to the time of writing.

Thus 16 bacteriologically proved cases of typhoid fever in all likelihood derived their infection directly or indirectly from Leonard E.

Baumann states that besides these 16 cases, there had undoubtedly been numerous slight infections not recognised as typhoid fever and where no physician had been called in.

The part played by carriers in maintaining the endemicity of enteric fever in country districts is further exemplified by the four cases reported by Watt, Medical Officer of Health, Aberdeenshire, 1909, in his report on the health of the County for the year 1908. My thanks are due to Dr. Watt for kindly supplying me with additional data. One of the four cases will be described in detail in Chapter IV. The histories of the other three cases are briefly as follows :—

Case 1.—A female carrier (aged 65 years) was discovered in connexion with a case of enteric fever on a farm "S" in Aberdeenshire in August, 1908. The person attacked was the farmer, who died, and the carrier was his housekeeper. This woman had had enteric fever in 1900 while a patient in a lunatic asylum (*see* account of carrier-cases in this asylum, Chapter V.). Soon after her recovery from the fever, which was a severe one, she was discharged from the asylum and went to reside at a farm "B" for a short time. At this farm an outbreak occurred and it was ascertained that this woman had been living at the farm when the first case occurred there. Subsequent outbreaks occurred in places where she was employed as cook or cook-housekeeper. Hotel in "R," 1901, October, three cases. Farm "A," 1902, April, three cases.

Also at another house where she was in service during 1902, the master and his daughter were attacked by an illness which in the light of subsequent events may be regarded as having possibly been typhoid fever, though not diagnosed as such.

Farm "O," 1905, January, two cases. Farmer and his son.

Farm "F," 1907, August, two cases. Male servants.

It is interesting to note that in view of the danger arising to the community from this woman, the Local Authority, with the consent of the Local Government Board for Scotland, resolved to grant her an allowance of five shillings a week until she reached the age when she would be entitled to a pension, on condition that she did not engage in any work involving the handling of food. This offer was accepted.

Case 2.—This female carrier (aged 43 years) was discovered in connexion with an outbreak of typhoid fever at a farm, involving three people, viz., two male servants and one female servant.

The daughter of the carrier was also attacked. A sister-in-law of the carrier (who was the farmer's wife) was living in the neighbourhood as a summer visitor and getting milk from her farm. She, too, with her two daughters developed enteric fever on their return to Aberdeen.

There had been previous outbreaks at this farm in 1892, 1897 and 1906. It was in 1890 that the farmer's wife had her primary attack just before her marriage. She and her husband took the farm in 1892 and the first outbreak occurred in that year, when the persons attacked were (1) the husband, aged 28 ; (2) a child, aged 13 months ; (3) a brother of the farmer who assisted with the farm work. There had been no previous cases at this farm.

In 1897 a man-servant and in 1906 a child of nine months were notified as cases of typhoid fever. Other illnesses had, however, occurred in the family, and these the medical attendant is now inclined to regard as having been probably typhoid fever.

Case 3.—This female carrier (aged 28 years) was the wife of a cottar on a farm. She had had enteric fever in August, 1907. In August and September, 1908, an outbreak of typhoid fever occurred in two adjoining cottar houses, one of which was occupied by the carrier. The persons attacked were the carrier's husband, and a child and a brother-in-law of her neighbour, who worked in a neighbouring town but spent the week-ends at the farm. The two houses were served by one privy which was in a very foul condition.

The bacteriological work in connection with these cases of Dr. Watt was carried out in the Pathological Department of the University of Aberdeen by Dr. A. R. Laing.

Irwin and Houston (1909) of Belfast have put on record a series of typhoid infections due to a female urinary carrier. She had had enteric fever seven years previously when she was a servant in a family at Liscard. Since her illness she had not enjoyed good health and complained of frequent headaches and gastric disturbances. After her recovery from the fever she took another situation in Liscard, but she had not been a month in her new post before the master of the house developed enteric fever. During the next two years she was in different situations but nothing of importance occurred. She then went to Liverpool and while serving in a family there, the son of her mistress developed enteric fever. Finally she went to Belfast, and while she was in service in that city four people living in the same house with her developed typhoid fever. In seven years therefore six people living in the same houses with her had contracted this disease. The urine of this woman contained large numbers of typhoid bacilli but the faeces were negative. Her serum gave an incomplete Widal reaction.

Recently Johnstone (1910–*a*) has reported to the Local Government Board on the association of carriers with endemic typhoid fever in a small Buckinghamshire hamlet. This hamlet (Jennet Hill) consists merely of a few cottages distributed in two groups, (1) a row of 10 dwellings known as Lailey's Row, facing upon a cul-de-sac off the main road between Stanford Dingley and Clay Cross, and (2) nine dwellings scattered irregularly along the same road in the direction of Stanford Dingley and in the direction of Clay Cross. The population of Jennet Hill itself is not more than 80 persons and if the neighbouring hamlets of Stanford Dingley, Clay Hill and Bucklebury Common be added, the population of Jennet Hill and its vicinity probably does not amount to 400.

During the period 1893–1907, 36 cases of enteric fever were notified from the rural district outside the vicinity of Jennet Hill with a population of about 15,000, while from Jennet Hill itself and its vicinity 26 cases were notified. During the period 1896–1907, 25 cases were notified from Jennet Hill and its vicinity while only 24 were notified from the remainder of the rural district. Jennet Hill itself, with a population of 80 people, supplied no fewer than 16 of these 25 cases.

The following table shows the number of cases of enteric fever year by year in the vicinity of Jennet Hill and in the remainder of the rural district of Bradfield respectively, during the period 1893–1907.

	Jennet Hill.	Rest of Rural District.
1893	1	1
1894	–	4
1895	–	7
1896	4	1
1897	8	2
1898	2	3
1899	5	2
1900	1	2
1901	–	6
1902	2	2
1903	–	–
1904	3	–
1905	–	3
1906	1	1
1907	4	2

A marked lack of correspondence between the two parts of the rural district is evident in most years, especially in 1894, 1895, 1897 and 1901, and although the total number of cases was small, the discrepancy suggested the existence of some influence which affected more particularly the neighbourhood of Jennet Hill.

In 1908 an enquiry was made by Dr. Johnstone with a view to ascertaining the cause of this special incidence of enteric fever on Jennet Hill, and in this connexion attention was at first mainly directed to water supply, milk supply, uncooked foods, mineral waters, and excrement-disposal. The evidence on these points must be consulted in the original, but it is sufficient to note here that no satisfactory solution of the excessive incidence of enteric fever on Jennet Hill was reached as the result of this enquiry.

The question then arose as to the possibility of carriers playing a part in the dissemination of enteric. Particular attention was directed to Lailey's Row, as only four of the ten houses composing this row had escaped the fever. Moreover, three of these four houses had but few inhabitants, and these of an age which would render them little susceptible to the disease. Circumstances connected with the occurrence of further cases of enteric fever in 1909, at Lailey's Row, and also in relation with a family that had until recently resided there, strengthened the suspicion that a carrier might be the disseminating agency of the disease. On March 27th, 1909, a boy of six, living a No. 8, Lailey's Row, developed enteric fever. This boy came to Jennet Hill in October, 1908, with his family, consisting of father, mother, and another brother aged 14. He had not been in contact with any known case of enteric fever and he had partaken of no suspicious food or drink.

The former occupants of No. 8, Lailey's Row had gone to a house about three miles away, and two persons were notified as suffering from enteric fever shortly after their return (March 1909) from a visit to this house.

In May a child of one year, residing at No. 9, Lailey's Row, took ill. She had been in this house since October 18th, 1908. The symptoms were obscure but not inconsistent with a diagnosis of typhoid fever, although examination of the stools in the third week after onset was negative.

In the meantime endeavours were being made to obtain samples of excreta (fæces and urine) and samples of blood from the inhabitants of Jennet Hill, and especially from those living in Lailey's Row. Great difficulty was experienced, but ultimately samples of fæces and urine were got from 18 persons, 14 of whom resided in Lailey's Row, also a sample of fæces from another person who formerly lived in No. 8, Lailey's Row. Blood samples were received from 15 persons, all residing in Lailey's Row. None of these persons were known to have had enteric fever. Six, however, gave a positive Widal reaction (1 in 100), while one gave a partial reaction (1 in 50). Four of the samples of fæces contained typhoid bacilli in large numbers, and repeated examinations of these were made:

A. Positive on May 13th, 1909, June 18th, July 3rd. Negative on June 4th and July 16th.
B. Positive on June 4th and July 16th.
C. Positive on June 4th and July 16th.
D. Positive on May 18th, June 2nd, July 9th. Negative on July 24th.

The sera of A, B and D gave a negative Widal reaction, while that of C gave a positive reaction.

The carriers A, B and C belonged to one family, being mother, father and son respectively. The mother presented no history of enteric fever. In 1888 she came to reside at Clay Hill, next door to the house in which a case occurred in 1893. She stated that, while at Clay Hill her husband B. and her two sons, one of whom was C., had enteric fever in April, 1891. In October, 1893, she removed to No. 9, Lailey's Row, but no cases were notified from Jennet Hill till July, 1896. There may have been unrecognised cases, however. From 1896 onwards cases continued to crop up in Lailey's Row and its neighbourhood, seven of them occurring in the houses next door to A.

The husband and son were both difficult persons to deal with, and there was doubt as to whether the samples of fæces received from them were samples of their own fæces or of the mother's fæces.

The carrier D. was a married woman who came to Lailey's Row in September, 1903. She had no history of enteric fever, nor was there any history of enteric fever in families with which she had lived as a domestic servant. In her own family no case of enteric fever is known to have occurred until October, 1906, when her husband was attacked. Two cases (see above) occurred in connexion with the house she has occupied since leaving Lailey's Row.

As only a limited number of examinations was practicable, it appeared possible that there might be other carriers at Jennet Hill; although the detection of two undoubted carriers as well as of two persons in whom the presence of this condition is not yet free from doubt, is sufficiently remarkable in so small a community.

Hilgermann (1908) reports a series of carrier infections in the district of Coblenz (seven carriers). One or two of these (house cases) may be referred to in this chapter.

An old woman of 71 years had long been suspected of causing fresh typhoid infections which broke out from time to time among the newly-imported servants. She had absolutely refused, however, to supply specimens of her fæces for examination. In 1907 another series of three cases occurred in her house. Samples were now insisted upon and obtained and her fæces gave a pure culture of typhoid bacilli. It was ascertained as the result of further enquiry that from 1895 to 1907 (she herself had had enteric fever in 1894) she had infected 15 persons altogether, viz., 1895, one case ; 1900, two cases ; 1902, two cases ; 1903, three cases ; 1905, four cases ; 1907, three cases.

The other six carriers, all of whom were middle-aged females, had each given rise to one or two infections only, and that too, although, in Hilgermann's view, they must have been in all probability excreting typhoid bacilli continuously. Many instances of intermittent infectivity of carriers are discussed throughout this memoir, but some data may be given here which tend to show that, though infections appear to be particularly frequent among persons coming for the first time into contact with carriers (*vide* Conradi's view, Chapter XII.), there are numerous cases showing that persons who have been a very considerable time in association with a carrier may suddenly contract infection.

Thus one of Hilgermann's carriers (female) after a lapse of five years after her primary attack, infected two persons, with one of whom she had had constant social intercourse. Another carrier (two years after her primary attack) infected her nephew who had been a year with her in the house. Also a case of marital infection occurred on this occasion through the agency of a female carrier. This woman had caused in 1904 a severe outbreak of typhoid fever in the Bavarian Pfalz. Three years later her husband contracted the fever. It appeared that this woman had managed to escape the continual supervision of the typhoid station by frequently changing her abode. She had apparently paid no attention to instructions as to disinfection.

Similar cases of marital infection have not frequently been recorded, and it would appear that in some instances at least the carrier-state may not have been acquired as the result of any obvious precedent infection. Mayer (1908) records such a case :—

On 23rd September, 1902, a servant girl in " D " entered on her service in the place of a sister who was ill with typhoid fever and in hospital. Later, on 5th October, this girl herself contracted typhoid fever. After some years she married a certain Johannes H. in " O." A small epidemic occurred in this hamlet, and there was an investigation, with the result that both Johannes H. and his wife were found to be chronic carriers. The husband, so far as he could remember, had never suffered from any abdominal disturbance, and the only conclusion that could be arrived at was, that he had become a temporary carrier as the result of association with his wife, who after her attack in 1902 continued to excrete typhoid bacilli. From being a temporary carrier he had developed into the chronic stage.

We have to bear in mind not only the possibility of marital cases like the above, but also of the occurrence of multiple carriers in a single family. Fornet (1909) who has recently employed this fact in support of his view that the carrier-state is an immunity-phenomenon, reports that in the course of the typhoid campaign in

South-West Germany, no fewer than 26 families were discovered in which at least two and sometimes three members were carriers.

I shall discuss in Chapter XII. Fornet's view as to the nature of the carrier-state, and the evidence he adduces in support of his view, but I may explain here that his belief is, that the carrier-state is the consequence of a symptomless re-infection, and that the bacilli shed by the chronic carrier may not necessarily be the lineal descendants of the strain which caused the primary infection. The multiple occurrence of carriers in families he would explain on the assumption that all were similarly exposed both to the conditions which rendered the primary infection possible, and to those which resulted in the second or symptomless infection, culminating in the carrier-state.

It would take too much space to refer even in summary terms to the extraordinary chain of typhoid cases which Mayer, G. (1910) has recently put on record in summing up his investigations into endemic typhoid fever in the Bavarian Pfalz during the period 1903–1908. Accompanying his report is a remarkable "Stammbaum" or genealogical tree in which 196 cases appear, all of which he attributes, recently or remotely to contact with one single case. The report must be consulted in the original, but it may be noted that 13 chronic carriers make their appearance in the table, and to these Mayer attributes prime importance in the spread of endemic typhoid fever.

Further interesting house cases and village cases have been reported by Seitz (1909) of Berlin, and Schumacher (1909-*b*) of Trier, the latter of whom has also contributed certain valuable data which will be referred to in the chapter on the statistics of carrier-infectivity (Chapter XIII.).

CHAPTER IV.

INSTANCES OF INFECTIVITY OF CARRIERS—*contd.*

Milk-Borne Cases.

In the foregoing section illustrative cases have been recorded of small outbreaks of enteric fever in individual houses or neighbouring groups of houses, due to association, of diverse kinds, with carriers.

The transmission of infection from the carrier to the healthy, has been in many cases through food-stuffs contaminated by the carrier, but the exact history of the infective agent in its passage to the new host, especially in the absence of cleanly personal habits either on the part of the carrier or his or her associates, cannot in all cases be satisfactorily determined.

In the milk-spread cases which have been recorded during the last few years, the infection has taken hold of comparatively large groups of cases and has been of a more or less explosive character.

In the cases now to be described the fountain head of the infection has proved to be some worker in the dairy from which the attacked consumers obtained their milk supply. I shall first refer to the outbreak recorded by Kossel (1907).

In the summer of 1906, 25 cases of typhoid fever occurred in a town of 60,700 inhabitants. These occurred in groups during the months of May (three cases), June (five cases), July (six cases), and August (11 cases).

Twenty-one out of the 25 cases obtained their milk from the same milk shop in "O." The milk was often drunk raw but in two cases it was boiled, the unboiled cream being used, however, in the preparation of ice pudding. The hygienic arrangements of the milk shop were not satisfactory but in spite of the improvements carried out in view of the earlier cases, a further outburst of cases took place in August. It was therefore suspected that the milk became infected at the farms supplying the milk shop. Three different farms supplied milk, but as one of these also sent milk to a large town "F," where, during November 1905–March 1906, numerous cases of enteric fever had occurred, suspicion naturally attached itself to this particular farm. The excreta of 15 persons at the farm were examined and on 29th October, 1906, a carrier was discovered in the person of a male who fed the swine and who also milked 5–10 cows daily. This person presented no history of typhoid fever. He had been employed for 20 years at the farm but he came originally from a village "B" where typhoid fever had been epidemic. Repeated examinations of the faeces of this man were positive and he was accordingly forbidden to milk. The expectation that no further cases would occur was not realised, for in May, 1907, a single case occurred in "F," also attributable to milk from this farm. On enquiry it was found that the swine-feeder, contrary to orders, was again employed in the dairy.

From the Strassburg Institute two outbreaks are reported by Kayser (1906-*b*). On 23rd, 29th and 30th March, 1905 five people living in different streets of the same quarter of Strassburg fell ill with typhoid fever, and one died. All had drank raw milk procured from a milk-seller "H." This man kept a book in which were noted the names of the farms supplying the milk through him to his various customers and from this book it was found that the milk consumed by the five cases came from two farms in the country district round Strassburg. On enquiry at these houses all were found healthy. One boy of 12, however, was found to have had an indefinite illness about six months before. His stools were examined on 25th April, 1905, and found to contain large numbers of typhoid bacilli. According to Kayser the milk might have become contaminated in various ways. He states that it might have been soiled directly by liquid manure from the cow-shed or the milk-pails may have been washed out with water from an unsatisfactory well situated close to the privy. All the other members of the household, including the mother, who suffered from gall stones, had negative fæces.

In the other farm the hygienic precautions taken in connexion with the milk-operations were certainly bad, but no evidence of infection could be found.

In the same year another small milk-borne epidemic occurred in Strassburg, involving 17 cases, with two deaths. The cases arose in two groups, *viz.*, eight between 5th and 23rd June, 1905, and nine between the commencement and the 26th of August. All had drunk raw milk coming from the same milk shop which sold mixed milk only. It was therefore, with some difficulty, and much touring among the farmers who supplied the milk that the enquiry could be prosecuted. In the course of the investigation, information was received that in a certain family D. a typhoid case had occurred in the past. Serum tests were made from the members of this family and that of Frau D. gave a positive reaction on 25th August. On 27th September the fæces of the members of this family were examined, and in those of Frau D. who was engaged in the milk business the typhoid bacillus was demonstrated. The milk from this particular house was ordered to be pasteurised daily before being offered for sale. No further cases occurred which could be brought into relationship with the dairy.

Chambers and Buchanan (1908), report an outbreak of milk-borne enteric in Glasgow associated with a carrier case. The notifications suggested two distinct waves of infection. The first was irregular and involved eight cases. After a lapse of six days during which no notifications were received, the second wave arose which was maintained unbroken till the end of the epidemic. In the authors' view this second wave presented the true characters of a milk-borne epidemic, viz., rapid increase of the daily notifications and of the numbers sickening simultaneously.

The irregular character of the first wave was explained by the discovery of a chronic carrier ; the secondary wave arose because one of the cases infected by the carrier was a member of a family supplying a portion of the milk. The carrier was a milk woman, who had a history of previous association with enteric fever. Sixteen years previously she had had enteric fever along with most of her family.

Lumsden and Woodward (1909) record a milk outbreak in Washington involving 54 cases. Of these, 33 received milk from a milk dealer A. and 21 from a milk dealer B. During the twelve months preceding this outbreak no cases had occurred in households supplied by A. while 7 cases had occurred in households supplied by B. At the beginning of this outbreak the prevalence of typhoid fever generally in the district of Columbia was on the decline. Only the sections of the city, supplied with milk by these two dealers, were involved, and in these sections the cases were confined to persons consuming milk from these two dealers. Among the customers of other dairymen supplying the same section of Washington, there was no unusual number of cases.

Forty-five households were affected and in eight instances two or more members of the same household took ill. There were 15 instances in which those members of the household who used milk freely were affected, while those who used none or used it sparingly, escaped. There were eight fatal cases, *i.e.*, a mortality rate of 14·8 per cent.

The following table shows the distribution of the cases according to age and sex.

Age-Groups.	Number of Cases.		
	Male.	Female.	Total.
0–4	4	1	5
5–9	5	8	13
10–14	5	3	8
15–19	2	2	4
20–24	3	3	6
25–29	1	6	7
30–34	3	1	4
35–39	1	2	3
40–44	0	1	1
45–49	0	0	0
50–54	0	0	0
55–59	0	0	0
60–64	1	1	2
65–69	0	1	1
Totals ...	25	29	54

Thus the majority of the cases were among children, about 50 per cent. of the cases being in persons under 15 years of age.

The following particulars with regard to the source of the milk delivered by the two dealers A. and B. are taken from an account of this outbreak in a Bulletin of the Hygiene Laboratory by Rosenau, Lumsden and Kastle (1909).

The dealer A. received milk from his own dairy farm and also a daily supply of 40 gallons from the dairy farm of a Mrs. X. Among the consumers of this mixed milk 33 cases of typhoid fever occurred with onsets of illness dating from 24th September to 22nd October.

The dealer B. received milk from 21 farms including a daily supply of 20 gallons from the farm of the same Mrs. X. Among his clients, 21 cases of typhoid fever occurred with onsets of illness dating from 24th September to 24th October.

Mrs. X. in addition to supplying the dealers A. and B. retailed 4 gallons daily to 11 households in one of which three cases of fever occurred with onsets of illness dating from 3rd October to 17th October.

The farm of Mrs. X. was therefore suspected as the source of the infection and as there had been no suspicious illnesses on this farm or in its neighbourhood, the possibility of a carrier suggested itself. Specimens of stools and urine from all the 13 persons on the farm were examined and all were negative except the fæces of Mrs. X. This woman had had typhoid fever 18 years before and had been in excellent health since. She had more to do with handling the milk than any of the others concerned. The milk was liable to be contaminated in other ways, as Mrs. X's. excreta were being deposited in an open privy on the place.

From 16th October, 1908, when Mrs. X. was discovered to be a carrier, the sale of milk from this farm was discontinued by order of the health officer. Eight days later (24th October) the last cases occurring among the customers of A. and B. had their definite onset of typhoid fever.

Judging by the dates of onset in the different cases, the infection appeared to have been more or less constantly in the milk for about one month. Mrs. X. had been in the dairy business for five years and it is remarkable that infection had not sooner occurred. No further specimens of her excreta could be obtained.

One of the most instructive milk-spread epidemics due to carrier infection is that reported by Scheller (1908).

For fourteen years (1894--1907) enteric fever had been prevalent on an estate in the neighbourhood of Königsberg. Thirty-two persons had been certainly infected, but there may have been many more, who had slight attacks only. The people affected had all to do with the estate and there were no cases notified in the village adjoining the estate. The evidence did not point to the water supply as the source of infection, and measures of house disinfection were fruitless in stemming the outbreaks. An extensive investigation was then undertaken from the carrier point of view.

Eight of the 32 persons attacked had their own cow, while 23 others received their milk regularly from the estate. In one case it was no longer possible to ascertain whether the family concerned had possessed a cow of their own or not. The total number of people who were supplied with milk from the estate was about 40, while the number who possessed their own cow was 140. With regard to the 8 cases of this latter series, it was ascertained that though they had their own cows, they applied for milk to the estate when the cows went "dry." The presumption was therefore strong that some relationship would be found between the milk supply from the estate and the cases of enteric fever. The milk that was not consumed on the spot was not sold, but was employed for the feeding of swine. Only the butter was sold to certain people and amongst these no cases of typhoid fever occurred. It was thus explained why the epidemic remained a local one.

The personnel of the dairy was then enquired into. One woman, Frau U., was found to have had typhoid fever 17 years previously. She had been engaged on the estate for about thirty years. From the estate books it was ascertained that she had commenced dairy-work 14 years before, and just a short time before the occurrence of the first case of typhoid fever. Her stools contained typhoid bacilli practically in pure culture, and they were occasionally demonstrable in the urine.

Bacteriological investigation was then made of the excreta of the 40 persons who consumed the milk from the estate, or who assisted in any capacity in the dairy work. Of these 40 persons no fewer than 18 were found to be carriers. One was the woman above mentioned. Of the others, four had had enteric some considerable time previously, while in the remaining 13 no record of a previous attack could be ascertained. The four carriers who had had enteric fever previously could have played no part in spreading the infection by milk, as they had never had anything to do with its preparation.

A large proportion of the 140 persons who received no milk from the estate was also examined but with negative results.

The point to be determined now was whether the woman already mentioned was a genuine chronic carrier or whether the other 17 carriers were also to be classed as such.

It may be noted here that these 17 persons were examined on three occasions at intervals of 12 days. Four were found to be passing typhoid bacilli both in the stools and in the urine, seven in the stools only and six in the urine only. The following test was made. Frau U. was discharged from her dairy-work and all the other carriers who were engaged in the milk business were strictly enjoined to wash their hands in lysol before milking. Further, the milk that was not used for butter was boiled before distribution. The urinary carriers also received urotropin. After a month under this regime all the carriers were again examined when it was found that Frau U. was still discharging bacilli in large numbers while repeated examination of the others (at 15 day intervals) gave negative results. From this it was inferred that Frau U. was a genuine chronic carrier while the other 17 were only temporary carriers.

Scheller believed on grounds which in the light of more recent knowledge, appear insufficient, that he had discovered a type of carrier in which the typhoid bacilli lead a true saprophytic existence in the intestinal canal and cause no disturbance whatever in the organism of the host.

Lentz had previously held the views (1) that a large proportion of these transitory carriers had really reacted to the presence of the typhoid bacilli by slight and possibly unnoticed symptoms, and (2) that those who did not have symptoms even of the slightest order, were still to be classed as "symptomless" typhoid cases in view of the fact that the agglutinin reaction in such cases rose to the same titre as in clinically typical enteric cases. With this latter statement of Lentz, Scheller does not agree as he had noted the absence of agglutinin reaction in some of these cases, while in others he had undoubtedly observed as high a titre as 1 in 2,000. The 17 temporary carriers who ceased to carry, as already described, Scheller preferred to classify in a new group of "Typhusbazillenzwischenträger" (intermediate carriers).

The view is open to criticism for the following reasons :—In the first place, 4 of these 17 carriers had had a definite primary infection, and the actual number of examinations was only three during a period of one month. Without further evidence, therefore, these four persons would not properly be classed as temporary carriers.

In the second place, with regard to the remaining 13 who had never had the slightest symptoms of typhoid fever, one would wish to know whether any of them developed the disease at a later period, as the possibility of a prolonged incubation stage is now well recognised. It is not disputed that this purely saprophytic growth may in some cases take place, but much fuller evidence is demanded before we can admit that such cases have never at any time exhibited symptoms either clinical or serological.

I shall now refer to an extensive series of outbreaks in Aberdeenshire associated with a female carrier who during a large number of years had managed dairy farms.

Watt, Medical Officer of Health, Aberdeenshire (1909), in his annual report on the health of the County of Aberdeen for the year 1908, records that 10 of the 38 cases of typhoid fever notified were traceable to carrier cases. I am indebted to Dr. Watt for furnishing me with particulars regarding the outbreaks associated with these carriers, one of whom is eclipsed only by the Folkestone carrier (*see* pp. 51–56), in so far as the number of her victims is concerned. This carrier, Miss S., a woman of 54 years, was found in connection with an outbreak of enteric fever on a dairy-farm "T" in Aberdeenshire during the summer of 1908. Three servants were attacked (two males and a female) two of whom died. Miss S., who with her brother occupied this farm, had had enteric fever in 1877 when she was 22 years of age. Enquiry showed that since that date a long series of typhoid cases had cropped up at the various dairy farms which she and her brother had managed during these 31 years, and also in Aberdeen, where milk-borne outbreaks traceable to these farms periodically occurred. The following data ascertained by Dr. Watt probably do not exhaust all the cases for which this woman was responsible :—

Farm I.	"G."	Autumn, 1877. Three cases. Brother of Miss S. Female servant, male servant.
		July, 1878. Two cases. Male servant, female servant.
		Spring, 1882. One case. Male servant.
		Summer, 1883. Two cases. Male servant, female servant.
		Summer, 1885. Two cases. Both male servants.
		August, 1897. Two cases. Male servants.
		November, 1898. Two cases. Female servant, male servant (died).
Farm II.	"N."	No cases.
Farm III.	"M."	August, 1900. One case. Male servant (died).
		January–March, 1901, Two cases. Male servants.
		September, 1902. One case. Male servant.
		September, 1903. One case. Maid servant.
		January, 1904. Two cases. Male servant, female servant.
		September, 1905. One case. Male servant.
Farm IV.	"T."	Summer, 1908. Three cases. Two male servants, one female servant (died).

Besides these cases on the farms, an outbreak in Aberdeen (November, 1898–January, 1899) involving 26 cases, was found to be connected with a dairy supplied by milk from farm "G." (*See* Report by Prof. Matthew Hay for the City of Aberdeen, November, 1899.)

From June, 1900–31st March, 1902, the milk from farm "M" had been sent to a certain dairy in Aberdeen and the following cases of typhoid fever were ascertained to have occurred among the customers of this dairy during the period 1898–1908.

1898. Four cases. August 1; September 1; December 2.
1899. Four cases. January 1; March 1; June 2.
1900. Seven cases. July 1; September 2; October 2; November 2.
1901. Eight cases. January 2; February 2; March 3; April 1; October 1.
1902. Nil.
1903. Nil.
1904. Four cases. February, April, August, November.
1905. Two cases. May, September.
1906. 1907. 1908. No cases.

Outbreaks in Aberdeen prior to 1898 would also appear to have been associated with farm "G," but the actual facts could not be ascertained. In any case it was certainly suggestive that this farm had been visited in September, 1878, 1882 and also in 1885, by the City medical officers of health in connexion with milk spread-outbreaks in Aberdeen. Probably, were all the facts known, it would be found that the number of cases directly or indirectly attributable to infection from Miss S. during the period 1877–1908 would not fall short of 100.

This carrier suffered from gall stones, for which operation was undertaken with a view to obtaining relief from colic troubles and possibly also with a view to ridding herself of typhoid bacilli. An account of the operation will be found in Chapter IX.

Richards, Medical Officer of Health, Croydon (1910), reports an outbreak of enteric fever in a large country house, associated with a carrier in the person of a milker. The cases arose in the following order:—

Case 1.—1903. The butler of a former tenant. Particulars not now ascertainable.

The present tenant took possession in 1906, and all went well till October, 1908.

Case 2.—Male, aged 26. October 22nd, 1908. Severe attack.

Case 3.—Female who lunched at the house on October 17th, 1908, and on November 4th, fell sick.

Case 4.—Kitchen maid. November 1st, 1908. Left the district after convalescence.

Case 5.—Boy. January 17th, 1910. He lived in one of the adjacent cottages.

Case 6.—Kitchen maid. March 11th, 1910.

On the occurrence of Cases 2, 3, and 4, the premises were thoroughly inspected, and certain defects in the drains made good. Water supply, consumption of shell-fish, and other possible sources of infection were exonerated, after careful enquiry. The excreta of Case 2 were examined with negative results, though his serum reaction was still positive. On the assumption that all the cases except the first had a common origin, suspicion could be attached only to those members of the household who were on the premises in 1908 and 1910. These were three in number, viz., the cook, who entered on her duties about March, 1908 ; a housemaid, who had lived in the house since May, 1904 ; and the milker, whose history will be detailed below.

Neither the cook nor the housemaid presented a history of a typhoid infection or association with enteric cases in other households. The excreta of both were examined with negative results.

The fæces of the milker, however, contained typhoid bacilli. This man never had enteric fever, but his wife had an attack in 1894, when they were living in another village. He came to this country house in 1902, and was milking at the time when Case 1 was infected in 1903. He left the district in September, 1906, but returned in October, 1908, when he entered on his present duties. Case 2 fell ill about October 22nd. It is important to note that between September, 1906, and October, 1908, this man was employed in a dairy in another district where a large amount of milk and butter was sold, but during that time there do not appear to have been any cases of enteric fever among the customers.

The following facts were ascertained with regard to the consumption of milk by the persons attacked :—

Case 1.—Cannot be traced, but as he was an indoor servant he doubtless consumed the milk supplied to the house.

Case 2.—Not only took milk, but was especially fond of cream.

Case 3.—Believed to have had cream in coffee and probably with fruit or tart.

Case 4.—Consumed both cream and milk.

Case 5.—Came to the house daily for milk.

Case 6.—Consumed milk and cream.

The milk was consumed mostly in the house, some being set for cream and some made into butter. The excess milk was disposed of in variable quantities to eight adjacent cottages, including the one occupied by Case 5.

That only six persons were attacked, although about 47 persons consumed the milk at various times, is largely accounted for by the fact that the quantity of milk consumed per head was small except in the large house, where most of the cases occurred. Further, as the history of other milk-spread epidemics due to carriers has shown, the opportunities of successful infection are notoriously intermittent. Dr. Richards suggests that the absence of infections during the milker's sojourn in a large dairy during 1906–1908 may have been due to the necessarily greater dilution of the poison which would thus act as a safeguard. The stools of this milker have since been examined by me on 19th May, 1910, with positive results. Those of his wife and daughter were, however, negative.

Recently a report by R. W. Johnstone (1910–*b*), on the recurrent prevalence of enteric fever in the Folkestone Urban District, has been submitted to the Local Government Board. The report is a most exhaustive one, and the details will be read with the greatest interest by all epidemiologists. Here I must confine myself to a brief survey of the enquiry.

The Urban District of Folkestone had at the Census of 1901 a population of 30,379, and in the middle of 1908 it was estimated to be 35,580. In the year 1901, Dr. Theodore Thomson had reported to the Local Government Board on recurrent enteric fever in that district, and had made careful enquiry into water supply, refuse and excrement disposal, sewerage and drainage, and the conditions prevailing in the various cowsheds, dairies and milkshops. He found that no satisfactory explanation of the repeated occurrences of enteric fever in Folkestone could be obtained by a consideration of the water supply and other sanitary conditions. He was able, however, to show that in certain years, viz., 1896, 1897, 1899 and 1900, milk was a prominent factor in the dissemination of enteric fever in Folkestone, while it may have been responsible for a small share of the fever in 1898. He also noticed that a certain milker had worked upon the three different milk farms which were associated with the dissemination of enteric fever in the years 1896, 1897 and 1899. At that time, however (1901), there was no knowledge of the typhoid-carrier, and Dr. Thomson had dismissed the coincidence with the remark "no milkman, even if uncleanly in his ways, can convey infective material unless the material be there to convey." Dr. Johnstone, who took up the enquiry in August, 1909, found that the facts elicited by Dr. Thomson with regard to water supply and general sanitary conditions still held good, and proceeded to enquire into the milk question.

Before detailing his results, I append the following tables (extracted from Dr. Johnstone's report) relative to the incidence of enteric fever in Folkestone from 1893 to 1909.

Table showing the number of deaths from enteric fever and the number of cases notified in the Urban District of Folkestone during the period 1893–1909. For comparison, similar figures are given for the neighbouring Urban Districts of Hythe (pop. 1901, 5,551), Sandgate (pop. 1901, 2,294), and Cheriton (pop. 1901, 7,091), and for the Rural District of Elham (pop. 1901, 6,813).

—	Folkestone Urban District.		Hythe Urban District.		Sandgate Urban District.		Elham Rural District.		Cheriton Urban District.	
	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.
1893	—	5	—	4	—	—	1	1		
1894	2	7	—	1	—	3	1	3		
1895	—	10	1	2	1	3	3	4		
1896	4	18	1	1	1	1	1	5		
1897	4	40	—	3	—	1	1	1		
1898	8	36	1	3	—	—	—	2		
1899	16	87	—	—	—	1	—	2	—	—
1900	5	50	—	—	1	9	—	1	—	—
1901	3	14	1	2	—	2	—	1	—	1
1902	6	26	—	2	—	2	1	2	1	3
1903	2	16	—	1	—	—	1	1	1	—
1904	1	7	—	3	—	2	—	2	—	—
1905	—	6	—	—	—	—	—	—	—	—
1906	—	11	—	2	—	—	—	1	1	1
1907	1	20	—	—	—	—	—	1	—	—
1908	—	9	—	—	—	—	1	3	—	—
1909	1	19	—	1	—	—	1	2	—	—
Total	53	381	4	25	3	24	11	32	3	5

From the figures it will be seen that enteric fever began to increase in Folkestone Urban District in 1896 and that it attained its maximum prevalence in 1899, subsiding in 1901 to show minor recrudescence in 1902, 1903, 1907 and 1909. No correspondence can be traced between the degree of prevalence of the fever in the other districts and that in Folkestone.

Table showing the incidence of enteric fever as regards the age and sex of persons attacked during the period 1896–1909.

Year.	-5	-15	-25	-35	-45	+45	Total at all ages.	Males	Females.	Over Age 15.	
										Males.	Females.
1896 ...	2	8	4	3	1	—	18	3	15	2	6
1897 ...	2	13	15	8	—	2	40	13	27	6	19
1898 ...	3	10	9	10	2	2	36	18	18	11	12
1899 ...	2	22	28	18	12	5	87	38	49	25	38
1900 ...	—	15	24	6	5	—	50	16	34	10	25
1901 ...	1	3	5	3	1	1	14	5	9	3	7
1902 ...	1	3	10	7	2	2	25	12	13	10	11
1903 ...	1	7	3	2	2	1	16	8	8	3	5
1904 ...	—	2	2	2	1	—	7	4	3	3	2
1905 ...	—	1	2	1	1	1	6	3	3	2	3
1906 ...	—	1	2	6	—	2	11	9	2	8	2
1907 ...	2	5	5	4	—	4	20	6	14	3	10
1908 ...	—	3	4	2	—	—	9	5	4	3	3
1909 ...	1	11	—	2	2	2	18	9	9	4	2
Whole period.	15	104	113	74	29	22	357	149	208	93	145

N.B.—In considering these figures it has to be borne in mind that at the Census of 1891, females to males in Folkestone were as 138 to 100, whereas in England and Wales the proportion was 108 to 100. At the Census of 1901 females to males in Folkestone were as 136 to 100 and in England and Wales as 107 to 100.

Results of Dr. Johnstone's enquiry :

During the period 1901–1909 the local Inspector of Nuisances had made careful inquiries which seemed to indicate that the milker referred to by Dr. Thomson in his report of 1901 was connected in other years with milk farms said to be associated with the dissemination of enteric fever. Samples of this man's excreta were now procured (fæces and urine) and submitted to bacteriological examination. The typhoid bacillus was isolated from the fæces on the five occasions on which samples were taken, viz., 31st August, 1909, 6th September, 1909, 11th September, 1909, 25th September, 1909, and 19th October, 1909. The urine was always negative. This milker N. was, therefore, a typhoid carrier. He was a man of about 60 years of age, enjoying excellent health. To his knowledge he had never suffered from enteric fever nor had his wife or any of his children while living with him. The only severe illness he was able to recall occurred about 30 years ago. At that time he had suffered from lassitude, lack of appetite, and general malaise, and though he was not bad enough to take to his bed, the illness had left a lively impression on him and his wife. Up to the age of 26, N. had been a sailor. He then took up farm work, but did not become a regular milker till April, 1893, when he came to a farm in the Elham Rural District, close to Folkestone.

From that time till August, 1909, he was constantly employed as cowman and milker on various farms near Folkestone, viz. :—

No. 1 Farm, April, 1893–October 11th, 1895, in Elham Rural District.

No. 2 Farm, October 11th, 1895-October 11th, 1896, in Elham Rural District.

No. 3 Farm, October 11th, 1896–October 11th, 1898, in Dover Rural District.

No. 4 Farm, October 11th, 1898–October 11th, 1902, in Elham Rural District.

No. 5 Farm, October 11th, 1902–October 11th, 1909, in Elham Rural District.

During N.'s stay at No. 1 farm there were five cases of enteric fever at the farm, viz., son and daughter of the farmer, a visitor, a maid-servant, and a charwoman.

At No. 2 farm there was one case of enteric fever while N. worked there.

At No. 4 farm two of the farmer's daughters had enteric fever while N. worked there.

Table showing year by year the number of cases of enteric fever notified in the Urban Districts of Folkestone, Hythe, Sandgate, and the Rural District of Elham during the period 1893–1909.

In separate columns are shown the number of persons who contracted the fever outside the district (imported cases) and the number of persons who lived in houses deriving their milk supply wholly or in part from a farm on which N. worked as a milker at the time of infection (N. cases).

—	Folkestone Urban District.			Hythe Urban District.			Sandgate Urban District.			Elham Rural District.		
	Total Cases.	Imported Cases.	N. Cases.	Total Cases.	Imported Cases.	N. Cases.	Total Cases.	Imported Cases.	N. Cases.	Total Cases.	Imported Cases.	N. Cases.
1893	5	—	—	4	—	—	—	—	—	1	—	1
1894	7	—	—	1	—	—	3	—	—	3	—	3
1895	10	—	—	2	—	—	3	—	3	4	2	1
1896	18	1	13	1	—	1	1	—	—	5	—	3
1897	49	3	20	3	1	—	1	—	—	1	—	1
1898	36	1	14	3	2	—	—	—	—	2	1	—
1899	87	2	66	—	—	—	1	—	—	2	—	2
1900	50	5	38	—	—	—	9	—	—	1	—	—
1901	14	3	10	2	—	2	2	—	—	1	—	—
1902	26	4	15	2	1	1	2	—	—	2	—	—
1903	16	5	7	1	—	—	—	—	—	1	—	—
1904	7	—	4	3	2	—	2	—	—	2	—	1
1905	6	1	—	—	—	—	—	—	—	—	—	—
1906	11	2	1	2	—	—	—	—	—	1	—	1
1907	20	6	3	—	—	—	—	—	—	1	1	—
1908	9	2	3	—	—	—	—	—	—	3	—	2
1909	19	1	13	1	—	—	—	—	—	1	—	1
Totals ...	381	36	207	25	6	4	24	—	3	31	4	16

For particulars with regard to the milk supply in the various years and the enteric outbreaks associated with it, the original report must be consulted.

In the 14-year period 1896–1909, 359 cases of enteric fever are known to have occurred in Folkestone Urban District, and of these 36 are known to have been imported cases. Of the remaining 323 cases, 207 or 64 per cent. are known to have had milk, within three or four weeks preceding their illness, from one or other of the farms on which N. was at the time acting as milker.

Interesting data were obtained relative to the incidence of enteric fever as regards the age and sex of the persons who had taken N.'s milk, and in the following table the figures are set forth :—

—	—5	—15	—25	—35	—45	+45	Total.	Males.	Females.	Over 15.	
										Males.	Females.
1896	1	6	4	2	—	—	13	3	10	1	5
1897	—	8	7	5	—	—	20	7	13	3	9
1898	—	7	4	3	—	—	14	6	8	2	5
1899	3	17	18	13	10	5	66	20	46	16	30
1900	—	14	16	6	2	—	38	11	27	5	19
1901	1	3	3	2	—	1	10	3	7	1	5
1902	1	2	5	2	4	1	15	9	6	7	5
1903	—	3	1	1	1	1	7	3	4	1	3
1904	—	—	2	2	—	—	4	2	2	2	2
1905	—	—	—	—	—	—	—	—	—	—	—
1906	—	—	1	—	—	—	1	1	—	1	—
1907	—	1	2	—	—	—	3	—	3	—	2
1908	—	2	—	1	—	—	3	2	1	1	—
1909	1	8	—	1	1	1	12	4	8	2	1
Total	7	71	63	38	18	9	206	71	135	42	86

A comparison of these figures with those relative to the cases which had not taken N's milk revealed fairly clearly that there was a disproportionate incidence of enteric fever on females and children in the group which had partaken of N's milk. This undue proportional incidence is in itself suggestive of milk-borne infection.

To sum up. N., a typhoid-carrier, worked at five different milk-farms as cowman and milker during the period 1893–1909. Enteric fever broke out at three of these farms during his stay there, and cases of enteric fever also occurred in the neighbourhood of all five of them. On the milk round of each of the five farms there was undue prevalence of the fever at intervals during the time N. worked there, and no such prevalence was observed either before his advent or after his departure. During the period 1896–1909 64 per cent. of the cases of enteric fever infected in Folkestone had been supplied with milk from a farm on which N. was working. Certainly milk from N's farm was supplied in two other districts for three months in 1901 without any known increase of enteric fever in these districts, and no cases of the fever could be attributed to N's milk during the year 1905, but as Johnstone remarks, N. may have been only intermittently infective, and even had his condition been one of continuous infection, it was not inevitable that the infection should always reach the milk-supply. "On the whole facts it may be concluded that for the past 14 years enteric fever has been spread in Folkestone Urban District mainly by milk, and that the milk was infected by N., a typhoid-carrier."

CHAPTER V.

INSTANCES OF INFECTIVITY OF CARRIERS—(*continued*).

Institutional Cases.

A large proportion of typhoid carriers have been found in institutions like asylums, workhouses, private schools, &c., where typhoid fever has been of endemic occurrence. In such establishments the intimate contact prevailing among the inmates must play a large part in facilitating the spread of infection from case to case. In lunatic asylums especially, the filthy personal habits of many insane people are naturally accentuating factors where a carrier or carriers are already in existence. Some attempts have been made to explain the presence of large numbers of carriers in lunatic asylums, and the hypothesis has been put forward that a psychosis is a predisposing factor to the carrying status.

There is no evidence in support of this view, but there is much to be said for the view held by some observers that the proportion of carriers discovered in institutions may ultimately prove not to be unduly large, since the facilities for thoroughly carrying out bacteriological investigations on a large scale are greater than is usually the case elsewhere, and correspondingly increase the probability of detecting the presence of carriers.

Several striking instances of endemic typhoid fever in asylums have been recorded in recent years.

Nieter and Liefmann (1906) of Halle, record observations made in a lunatic asylum at "M," containing 900 female patients, in which, for a number of years, there had been repeated outbreaks of typhoid fever. These outbreaks had always occurred in small groups. Most of the cases were confined to one building containing about 250 inmates. After an examination of the convalescent cases had yielded no carriers, they proceeded to examine all the inmates in this block. In one case of acute dysentery the B. typhosus was found in the fæces along with B. dysenteriae (Flexner). The association was explained by the fact that cases of dysentery and of typhoid fever were isolated in the same block, and it seemed likely that the dysentery patient had got secondarily infected with the typhoid bacillus. Her serum agglutinated B. typhosus in 1 in 100, and B. dysenteriae (Flexner) in 1 in 800.

In another dysentery case they found the typhoid bacillus in the stools but not the dysentery bacillus, and at her death numerous old typhoid cicatrices were found in the small intestine, while the lower bowel showed chronic catarrh. The gall bladder was full of large and small stones, and the typhoid bacillus was present in pure culture in the bile.

Other five carriers were discovered, making seven in all out of a total of 250 inmates. This makes a percentage of 2·8 per cent., which is much higher than that obtained by searching the neighbourhood of typhoid patients (viz., 0·5—0·8 per cent.). All the carriers were women. After isolation of all the carriers it was hoped that no further cases would occur, but this hope was not realised.

In a further report by Nieter (1907) on this asylum, it is stated that four more carriers were discovered during the late summer of 1906, thus making a total of 11. About the latter part of December and the beginning of January, 1907, three new cases of typhoid fever occurred among nurses employed in this same block of 250 inmates, where the seven carriers had been discovered. Another investigation was made and another female carrier was found. This person had never had clinical symptoms of typhoid fever. Paratyphoid bacilli were also present in her fæces.

After an interval of 4–5 months, three fresh cases occurred in another building in which two carriers had been previously found. In this room was a patient who two years before had had typhoid fever. During the first investigation this woman's excreta had been negative, though she had given a positive Widal reaction.

A second examination was now made and this woman was found to be passing typhoid bacilli in her stools. Thirteen carriers had been discovered in this asylum up to 1907.

Nieter mentions that in other asylums several carrier cases had been isolated, viz., Hordt (13 female carriers), Klingemünster (two carriers), Saargemund (two carriers), Merzig (one carrier).

Friedel (1906) reported a striking instance of institutional typhoid fever due to carrier infection, in which foodstuffs, in all probability, formed the intermediate link between the carrier and the new hosts. The institution affected was the Provinzial-Heil-und Pflegeanstalt at Andernach in the Coblenz district. On 1st September, 1905, this asylum contained 650 persons distributed thus :—

Group	A.	Patients, male and female ...	500
,,	B.	Nurses, 36 male and 36 female ...	72
,,	C.	Staff, various	20
,,	D.	Families in detached villas ...	45 persons.

All these groups had the same water supply. Groups A, B and C obtained food from the main kitchen, while the families in group D had their own kitchens.

Before 1901 only occasional cases of typhoid fever considered to have been due to outside infection had occurred. In 1901 two outbreaks arose in different blocks, viz., in May, nine cases (one physician, five male nurses, three male and one female patients), and in October, 12 cases (nine female nurses, one male nurse, one male and one female patient). In May, 1905, seven cases (one male nurse, six male patients), occurred in one male block.

Between 1901 and 1905 there were seven isolated cases, of which six were believed to have contracted the infection inside the building.

In these outbreaks the water supply could not be called in question. The possibility of already infected food coming from outside the institute was ruled out. Suspicion accordingly fell on the kitchen. The food was prepared here and then distributed to the various blocks.

Accordingly all those persons engaged in the dairy or kitchen who were ascertained to have had typhoid fever were examined, but with negative results.

In September, 1905, another outbreak occurred with 35 cases, 8 male nurses, 8 female nurses, 4 males on the staff, 5 male and 10 female patients, but none belonging to Group D.

The fæces and urine of all engaged in the kitchen, wash-house, scullery, dairy and laundry were now examined, whether they had had typhoid fever previously or not, and one female imbecile (65 years), who had been six years in the kitchen was found to be a carrier. She had never had typhoid fever, at least in the institution.

Her serum gave a negative Widal reaction. The chief duty of this woman was the cutting up of potatoes for potato salad and pieces of meat for Fleischsalat and various other salads. There is undoubtedly great danger attached to the consumption of such raw food material which has been contaminated during its preparation, or of cooked material which becomes contaminated before its final consumption in the cold state. In both cases cold storage is indicated as tending to lessen the likelihood of rapid multiplication of the B. typhosus and, therefore, of risk of infection to the consumer.

Ledingham, A. & J. C. G. (1908) have recorded the results of an investigation into the cause of recurring enteric fever in a lunatic asylum in Scotland. This asylum in 1908 contained 92 male and 53 female patients, 8 male and 6 female attendants and 4 female servants. Since 1893 there had been a succession of small outbreaks of typhoid fever in this asylum, the female inmates being principally affected. Also similar outbreaks had occurred periodically in a smaller detached building containing 32 female patients, and situated about five minutes' walk from the main building. Prior to the adoption of the Notification Act in 1891, no authentic records of typhoid fever in this asylum were available. The annexed table shows the incidence of typhoid fever in successive years since 1893, when the first case was notified :

Year.	Total No. of Cases.	Month of Notification.	Main Asylum.	Small Asylum.
1893 ...	2	Mar., July	2	0
1894 ...	0	... ...	0	0
1895 ...	6	July (2), Aug. (3), Dec. (1)	1	5
1896 ...	2	Apr., May	2	0
1897 ...	1	Nov.	0	1
1898 ...	0	... ...	0	0
1899 ...	4	Aug. (1), Nov. (3)	4	0
1900 ...	3	Jan., June, Aug.	1	2
1901 ...	2	Feb., May	2	0
1902 ...	0	... ...	0	0
1903 ...	4	Feb., Mar., Apr , Oct. ...	4	0
1904 ...	3	June, July, Aug.	3	0
1905 ...	0	... ...	0	0
1906 ...	1	April	1	0
1907 ...	3	Feb., Apr., Oct.	2	1
Total ...	31	... ...	22	9

It will be seen that 31 cases in all occurred and 24 of these were females, viz., 21 female patients, three female attendants, six male patients and one male attendant. Nine of the cases proved fatal. The cases arose at no special season of the year, but in individual years the notifications followed each other as a rule very closely. During 1894, 1898, 1902 and 1905 no cases were notified.

The mysterious cropping up of typhoid cases had been a source of great anxiety to the authorities, who had done everything in their power to make the sanitary arrangements of the institution as complete as possible. Repeated analyses of the water supply, both chemically and bacteriologically, threw no light on the etiology of the outbreaks. The sewage was conveyed direct to the sea and the dairy arrangements were in excellent order. The same water supply served several families residing on a farm near the source of the supply, but at no time had cases of enteric fever occurred among them. It was accordingly determined to search for carriers among the inmates. In February, 1907, a start was made by testing the Widal reaction in a group of 43 female patients, and six of these, two of whom had had enteric fever previously (in 1903 and

1904 respectively), gave a well-marked reaction. In the course of this work, however, A. L. contracted a severe attack of typhoid fever, and nothing further was done in the matter till September, 1907, when it was proposed to examine bacteriologically the stools of all the female patients in the asylum. Three female typhoid carriers were thus discovered. The first, G., had been in the asylum (main block) since 1896. She was 35 years of age, and there was no record of a primary infection. Her personal habits were exceedingly filthy. She had not, however, been employed either in the laundry or kitchen. Her serum gave a Widal reaction in a dilution of 1 in 200.

The second carrier, McC., had been in the small detached building since 1895, when she had had an attack of typhoid fever. She was about 60 years of age and was able to walk about and do a little work, but had never been employed in the kitchen. Her habits were cleanly, and occasionally she had assisted in the laundry. Her serum gave a Widal reaction in 1 in 200. It has to be noted that, though there are two separate blocks, much communication goes on between them. The dinners are cooked at the main block and brought over to the smaller block. The clothes are washed also at the main block, but patients from the small block would sometimes assist.

The third female carrier, C., was about 30 years old and had typhoid fever in 1904. She was not of dirty habits, but had never been employed in the kitchen or laundry. Her serum gave a marked Widal reaction.

These three females were isolated as soon as they were discovered to be carriers, and since November, 1907, they have remained in the isolation block with a special attendant.

Since October, 1907, the asylum has enjoyed complete immunity from typhoid fever. There have been 42 females admitted into the asylum during this period and it is proposed to examine all these bacteriologically, and in future to examine each new patient on admission into the asylum.

Eccard (1910) records a very similar experience of endemic typhoid fever in an asylum at Frankenthal in the Pfalz district. This institution contains 650 mental cases in one department, and 250 bodily-sick cases in another. Long before 1901 enteric fever had been of endemic occurrence, but since that year infection had occurred among the women only, the chief focus of the disease being the female block containing 110 " unclean, stupid and noisy " women.

In 1903 this block was gone over with the result that three female carriers were found. These had been housed for a long time in this department and had never been ill. An isolation barrack was put up for them and to this the typhoid cases were also transferred.

In spite of these precautions fresh cases cropped up and the conclusion was come to that some carriers, probably of the intermittent variety, had been missed. This proved to be the case. The investigation was repeated and in June, 1906, another female carrier was found. In December of the same year a fifth carrier, in May, 1907, a sixth, and in July, 1907, a seventh carrier was revealed. The examination of the stools and urine of these last three carriers had given negative results on previous occasions. The stools and urine of the carrier who was discovered in December, 1906, had been examined previously with negative results no fewer than five times, and still it was she who apparently kept the epidemic going, for after her isolation the occurrences of fever ceased, nor have there been any further cases in the three years up to 1909.

The following table shows the incidence of typhoid cases according to months and years from 1901 to 1st August, 1909 :—

Years.	Months.												Totals.
	Jan.	Feb.	Mar.	Apr.	May.	Jun.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	
1901 ...	—	—	—	2	1	1	—	—	—	1	—	—	5
1902 ...	—	—	—	1	1	—	—	—	—	1	—	—	3
1903 ...	—	—	—	—	—	—	—	—	1	4	—	—	5
1904 ...	1	—	1	1	—	—	—	—	1	—	—	—	4
1905 ...	—	—	—	—	—	—	—	—	1	—	—	1	2
1906 ...	—	—	—	—	1	—	—	—	*1	1	—	—	3 − 1
1907 ...	—	—	—	—	—	—	—	—	—	—	—	—	0
1908 ...	—	—	—	—	—	—	—	—	—	—	—	—	0
1909 ...	—	—	—	—	—	—	—	—	—	—	—	—	0

* Imported case.

Reformatory Case.

The female carrier case reported by Davies & Walker Hall (1908) is so well known in this country that I need only refer to the most important features of the outbreaks for which she was responsible. In November, 1906, an outbreak of typhoid fever occurred in an Inebriate Reformatory near Bristol. This institution then contained 240 inmates and 24 resident officers. There had been no visitation of typhoid fever since the opening of the institution in 1899. No cases of typhoid fever existed in the neighbourhood at the time of the outbreak. In September, 1906, a kitchen helper took ill, and in November three more cases occurred in which the suggestion was that the infection was conveyed by milk. One was a female inmate who received an extra allowance of milk daily, as she was nursing ; another was the gardener's wife who lived in a detached cottage and received only milk from the institution. The third was an outdoor policeman who received an allowance of milk for his tea. No explanation of the initial case was forthcoming. Fresh outbreaks occurred in May, 1907, and continued up to

November, viz., May, 1 case; June, nil; July, 6; August, 9; September, 1; October, 2; November, 4, *i.e.*, 23 altogether.

The evidence in the 1907 cases was still stronger against the milk which evidently had some opportunity of being contaminated after sterilization. It was learned that an inmate, Mrs. H. (50 years), employed as cook and dairymaid in the institution had suffered in January, 1901, from typhoid fever. She had been admitted to the Reformatory in April, 1906, and was employed in kitchen work up to October 13th, 1906, when she was entrusted also with the dairy work which she continued up to November, 1907. At Dr. Davies's suggestion she was excluded from kitchen and dairy work on November 13th, and 12 days afterwards, November 25th, the last case of the series occurred. On November 18th, 1907, her serum gave a negative Widal reaction, and the stools and urine were typhoid-free. On November 29th, 1907, the stools also proved negative, but a positive result was obtained on December 20th, 1907. Further examinations on January 20th, February 14th, February 18th, and March 14th were, however, negative. The urine was always negative. The fæces again proved positive on April 14th, 1908. (For further data on the bacteriological examinations of the excreta of this case *see* Chap. XII.) The milk after sterilization was stored in the dairy whence it was measured out for the various blocks by means of a hand-dipper. All the milk passed through her hands. It is evident, therefore, that the milk which was shown to have been the agency by which the fever was disseminated could readily have been infected by Mrs. H.

Enquiry into the past history of this carrier showed that she had been in all probability responsible for enteric outbreaks in at least two other institutions.

In May, 1904, typhoid fever broke out in the Grove House Home for Girls at Brislington, near Bristol. This institution was then occupied by 36 girls (5—15 years) who were boarded out by the Bristol Guardians. From May to the end of September, 26 persons in the institution developed enteric fever. Eight suspicious cases also occurred. Two cases ended fatally.

There had been no typhoid fever in the district for months and the sanitary arrangements of the institution could not be impugned. All attempts to combat the spread of the disease by disinfection, boiling of milk, &c., were fruitless. It appears that Mrs. H. had on February 3rd, 1904, been transferred from the Bristol workhouse to the kitchen at Grove House, Brislington. Her duties were to assist in the kitchen and to cook, and all the milk, which was boiled, passed through her hands. It is notable that she left Grove House Home on September 2nd to take a private situation, and the last case in the institution was notified twenty days later, viz., on September 22nd, 1904.

It was further ascertained that on March 4th, 1905, this carrier became cook at a children's home where there were 30 girls. Nothing happened till May 8th, 1905, when one of the girls developed enteric fever for which no cause could be found. The cook had left at the end of April. No further cases occurred.

I conclude this chapter with certain general observations regarding the occurrence of carriers in asylums and similar institutions. As I have mentioned, the higher frequency of carriers in asylums as compared with that found in the neighbourhood of typhoid cases elsewhere, has been attributed to a psychosis predisposing typhoid-sick lunatics to this condition.

There are, however, certain considerations which tend to discountenance the need for adopting such an explanatory hypothesis as this. In the first place much greater facilities are afforded to one chronic carrier of unclean habits, of infecting her associates, who may then act as temporary carriers or who may become chronic carriers. In the second place, as Eccard suggests, a lunatic typhoid-convalescent of filthy habits is probably always re-infecting herself and so develops into the chronic type. This view has been applied in a wider sense to all chronic carriers by Fornet, who as I have said (*vide* Chapter III.) maintains that the carrier state arises from a symptomless re-infection and is an evidence of immunity in the sense of Wassermann and Citron.

CHAPTER VI.

Instances of Infectivity of Carriers—(*continued*).

Army Cases.

The association of carriers with occurrences of typhoid fever in army barracks, &c., presents features similar to those met with in civil communities, but it seems advisable for facility of reference to discuss the subject of army carriers in a separate chapter.

The most extensive work which has so far been done in this connection has emanated from the Central Research Institute at Kasauli in India, where an enquiry into the cause and spread of enteric fever was set on foot in 1906 under the guidance of Lieut.-Colonel D. Semple and Captain E. D. W. Greig.

Their first report (Semple and Greig (1908)) on the investigations carried out appeared in 1908 and abundantly confirmed the experiences of the typhoid-stations in South-West Germany. I have referred in a previous chapter (Chapter II.) to certain statistical results collated by them in connexion with the bacteriological examination of convalescents, and it now remains here to record details of the regimental outbreaks of typhoid fever for which carriers were found to be responsible.

The first outbreak occurred in a detachment of the Bedfordshire Regiment at Kasauli. During the month of August, 1907, 5 cases of enteric fever occurred in this detachment. Two barracks were involved; each of these was divided into four wards; the cases arose in different rooms and there had been no direct personal communication between the patients. The food supply of the two barracks came from a common cook-house and the latrine and urinal were also in common. As the cases were confined to one detachment the general water and milk supply could be eliminated.

Examination was therefore made of the blood, urine and fæces of all the cooks and contacts of this unit with the result that one cook belonging to this detachment was found to be excreting large numbers of typhoid bacilli in his fæces.

The carrier was at once isolated and no further cases occurred.

A definite history of enteric fever in this carrier could not be elicited, but there was a probability that a severe attack of ague (31st December, 1897—10th March, 1898) lasting seventy days may have been an unrecognised typhoid infection. It was also noteworthy that, according to the health registers, from 23rd May to 3rd July, 1899, he had been treated for abscess, which may have been also of typhoidal origin. His Widal reaction was negative, agglutination taking place only in a dilution of 1 in 20.

The second outbreak occurred in the Scottish Rifles (Cameronians). As a result of the examination of a large number of healthy soldiers at Meerut from the different units stationed there, one man belonging to the Scottish Rifles was found to be a carrier. He had never suffered from enteric fever, but had been a nursing orderly on enteric cases. I have already noted (Chapter II.) that these orderlies not infrequently excrete typhoid bacilli without showing any obvious symptoms and are liable to become chronic carriers.

The connexion between this carrier and the particular cases of enteric fever could not be so clearly established as in the first outbreak, but the fact that seven out of the ten cases examined at Meerut came from this particular unit of the Scottish Rifles was considered very significant.

Semple and Greig, in view of the danger that nursing orderlies may contract the disease so slightly as not to report themselves sick, but may nevertheless become carriers, suggest that a special permanent body of trained men should be formed to nurse enteric patients.

An extremely interesting case of carrier infection in an artillery barracks at Wesel has recently been reported by Niepratschk (1909). During the years 1904–1908 this barracks had been, as the author expresses it, a "veritable typhoid-house." Its hygienic conditions were excellent and no source of infection could be traced. Since October, 1902, it had been occupied by the artillery regiment, and on January, 1904, the first case of typhoid fever occurred. During the five years 1904–1908, there had been 31 cases of typhoid fever, of which six ended fatally and four led to invalidity. The cases occurred as follows :—

1904. One case. (January.)
1905. Seven cases. (February, May, October (3), November, December.)
1906. Two cases. (April and October.)
1907. Twenty cases. (Ten between January and June. Ten between September and November.)
1908. One case. (February.)

The first two cases were attributed to infection contracted during Christmas leave, and the third case was also in all probability imported. For the other cases no probable source of infection was forthcoming. Neither in the civil population nor in the other troops of the garrison was the typhoid incidence high. The opinion that water played any part in the spread of the fever had to be abandoned after careful attention paid to the water supply had yielded no result. The hypothesis that a carrier might be at the root of the trouble was then tested, and a commencement was made with an examination of the fæces and urine of the lessee and assistants in the canteen. The cook (a female of 55 years of age) had had enteric fever two years before, and in August, 1907, had suffered from a febrile attack pointing to gall-bladder trouble. Repeated examinations of her fæces and urine were, however, negative, and she gave an absolutely negative Widal reaction. The examinations of the stools and urine of all the other assistants were negative.

Then began a systematic examination of the " Unteroffizieren " of the individual batteries. On 23rd February, 1908, Sergeant B. was found to be excreting large numbers of typhoid bacilli in his urine. No other carriers were found.

The history of this man was as follows :—In the spring of 1901 (5th March to 29th April) he had had a severe attack of enteric fever while serving in a regiment of Dragoons at Tilsit. His urine during the fever had been free from albumin. On 29th April he was sent home to recruit, but eight days later he had a severe relapse and for eight weeks could not be transported. On 20th June he returned to the regimental hospital and remained there till 8th July. At the close of his three years' service in October, 1901, he spent a year at home. On October 1, 1902, he joined the 15th Ulan regiment at Saarburg, where he remained till 1st October, 1903. He then returned home, but enlisted again on 15th June, 1904, in the 1st " Trainbataillon," at Königsberg. With this regiment he remained only one year, and on 15th June, 1905, joined the Artillery regiment at Wesel.

Some suggestive facts were later ascertained with regard to typhoid outbreaks in other regiments to which this man belonged before coming to the Wesel garrison. Thus in the year in which Sergeant B. was in a " Trainbataillon," one case occurred, while during the previous three years there had been no cases in that regiment. Also during his service in an Ulan regiment, 15 cases had occurred in 1903. None had occurred before his arrival and none after his departure.

Sergeant B. was a robust, healthy man of 31. His urine was slightly acid, showed slight cloudiness, but contained no albumen. No other formed elements were present. It was calculated that the urine contained two-and-a-half millions of typhoid bacilli per cubic centimetre. The strain was markedly avirulent for guinea-pigs, a fact which was in striking contrast with the severity of infections caused by it. When first isolated it would not kill guinea-pigs of 250 grms. weight in doses under a quarter of a whole agar culture. By passage, however, the lethal dose was lowered to one quarter of a loop. His serum gave an incomplete Widal reaction (1 in 50 positive in two hours, 1 in 100 negative in two hours at 37° C), and the bactericidal power of the serum was also very low. The fact that the virulence could be raised by passage through the guinea-pig suggested to Niepratschk that a similar process might take place by passage through human beings. Thus cases with only slight symptoms might give rise to infections of a severer type. There was the further possibility that the strain might undergo temporary alterations in virulence. In Niepratschk's view, however, one thing was certain, viz., that since October, 1905, the typhoid outbreaks in the artillery barracks could be traced only to this carrier. Among the other troops in Wesel and in the civil community there had been only a few cases.

In the following table are given the figures of typhoid incidence in the artillery department compared with that in other garrisons at Wesel and in the civil population :—

TYPHOID INCIDENCE PER 1,000.

—	1904.	1905.	1906.	1907.	1908.
Affected artillery detachment...	2·74	19·18	5·48	54·79	2·74
Other garrisons	0	1·07	0·53	0·26	0
Civil population	0·36	1·18	0·2	0·61	0·1

The table shows very clearly the heavy incidence of typhoid fever in the artillery detachment.

All three batteries of artillery were equally affected (1st Battery, 11 cases ; 2nd Battery, 9 cases ; 3rd Battery, 11 cases), and not Sergeant B.'s battery only. B. stated that he was in the habit of passing his urine only in the latrine. He may, however, in Niepratschk's opinion, have urinated occasionally in the stalls, so that the persons who had to change the straw or clean the stalls may readily have soiled their fingers or the soles of their boots with the typhoid bacilli. When they came to clean their boots the same danger was present. It was significant that the shoemaker who mended Sergeant B.'s boots took typhoid fever. Out of the 28 cases only two " Unteroffizieren " (the class to which Sergeant B. belonged) developed typhoid fever, and it is suggested that their immunity arose from the fact that they did not brush their own boots.

Sergeant B. was isolated for two years, and no further cases occurred in the regiment. The treatment adopted with apparent success in the case of Sergeant B. will be referred to in the chapter on urinary carriers.

The following regimental cases at Kilworth (Ireland) are reported for convenience in this chapter although the carrier in question was a civilian. The infection was transmitted by milk contaminated by the carrier. Dorgan (1910) has recently published a brief account in the Journal of the Royal Army Medical Corps, and from this I quote the following particulars :

Twenty-four cases in all occurred among the various regiments stationed at Kilworth during the period May to August, 1909, and the following table shows the dates of onset with remarks relative to the origin of the milk supplied to the respective messes affected :—

No.	Name.	Regiment.	Onset.	Remarks.
1	Captain C. ...	E. Yorks...	24.5.09	Inspector of musketry. Had tea in garrison mess about end of April.
2	Lieut. D. ...	Sherwoods	8.7.09	Lived at garrison mess. (O'Ns. milk.)
3	Pte. R. ...	Sherwoods	17.7.09	Waiter at garrison mess. (O'Ns. milk.)
4	Lieut. P. ...	S. Lancs....	12.7.09	Officers at a detached mess and used O'Ns. milk. Privates acted as waiters.
5	,, M. ...	,, ...	17.7.09	
6	Pte. C. ...	,, ...	15.7.09	
7	,, A. ...	,, ...	17.7.09	Died.
8	Capt. B. ...	R. M. F. ...	26.8.09	Regimental mess. O'Ns. milk.
9	Lieut. H. ...	R. A. M. C.	23.8.09	Garrison mess. O'Ns. milk.
10	,, W. ...	R. W. F....	24.8.09	
11	Lieut. M. ...	R. M. F. ...	28.8.09	Regimental mess. O'Ns. milk.
12	,, D. ...	,, ...	31.8.09	
13	,, S. ...	,, ...	1.9.09	
14	,, H. ...	,, ...	31.8.09	
15	,, E. ...	,, ...	1.9.09	
16	,, C. ...	,, ...	30.8.09	
17	Capt. S. ...	,, ...	3.9.09	
18	Pte. D. ...	R. M. F. ...	16.8.09	Kitchen man (officers' mess). Had been ill a few days on duty and may have infected the mess.
19	Pte. J. ...	R. M. F. ...	2.9.09	Mess waiter.
20	Pte. S. ...	R. M. F. ...	30.8.09	Regimental duty : the only case that cannot be traced to the milk.
21	Lance-Serg. S.	R. M. F. ...	23.8.09	Died.
22	Serg. B. ...		21.80.9	Sergeants' mess. O'Ns. milk.
23	Pte. G. ...	Conn. Rang.	27.8.09	Mess waiter. Admits drinking unboiled milk.
24	Child " B."...	R. E. ...	19.8.09	Visited camp and drank milk at O'Ns. farm on Aug. 10.

In all cases the milk which contrary to explicit regimental orders appeared to have been consumed in the unboiled state by the soldiers attacked came from the same farm occupied by a farmer O'N. The members of O'N's. household were examined by the Widal test and one girl, a dairymaid, was found to give a definite reaction in 1 in 100. After some difficulty, specimens of her excreta were obtained, and the B. typhosus was isolated from her urine. This woman had had enteric fever in 1903 when she was employed at this farm. Six months after her attack she returned to her duties there, when the farmer and his child were attacked. In 1906 she was employed in a similar capacity by a farmer at "G" near Kilworth. Soon after her arrival the farmer's child became ill, then the farmer himself and his wife, and also a labourer's wife and child. Two other children who had been at a boarding-school returned home on vacation, and both developed typhoid fever. On February 1st, 1909, she returned to reside at O'N's farm, but since November, 1908, she had worked there at odd times. Two sisters of O'N. died in December, 1908, of an acute fever which was since believed to have been enteric. Also a carpenter working in the camp and drinking O'N's milk contracted typhoid fever on February 7th, 1909. It was further ascertained that another married sister of O'N., who lived on a farm about a mile away was ill with enteric fever in September, 1909. There was much communication between the two houses. Also in the same month a civilian post office clerk contracted typhoid fever. O'N. supplied this post office with milk.

Including the civilian cases, this urinary carrier appears to have been responsible for about 40 cases of typhoid fever altogether.

Tsuzuki (1910) has recently reported on the occurrence of carrier cases in a division of the Japanese Army. The division in question (the XVth), which was formed during the war of 1904–5, was first attacked by typhoid fever while in Manchuria. After the war it remained in Korea till February, 1907, when its quarters were transferred to Narachino about 30 kilometres from Tokio. In October, 1908, it was transferred to Toyohashi. During 1906, 1907, and 1908 the numbers of enteric cases were respectively, 373, 211, and 64, being distributed as follows :—

Month.	1906.	1907.	1908.
January	15	1	6
February	2	2	3
March	8	1	7
April	—	—	9
May	4	1	14
June	16	1	16
July	44	32	—
August	54	67	4
September	87	87	4
October	80	11	1
November	50	8	—
December	13	—	—
Totals	373	211	64

The bacteriological examinations were carried out during the eight months, March–October, 1908, and there were examined—

(1.) All persons who had previously had typhoid.
(2.) Healthy men in companies where a new case had occurred.
(3.) Persons occupied in the kitchens.
(4.) Convalescents.

In all 5,109 examinations were performed of 2,785 persons. The results of this investigation are detailed in Chapter II. A total of 11 carriers was discovered, viz., six who had previously had enteric, two who had no record of typhoid, and three convalescent carriers. There was, therefore, in Tsuzuki's view little doubt as to the origin of the outbreaks in this division both during the campaign in Manchuria and in their quarters in Korea. Since the carriers were discharged from the regiment, the garrison at Toyohashi has displayed a satisfactory bill of health.

CHAPTER VII.

PATHOGENESIS OF THE TYPHOID-CARRYING STATE, WITH PARTICULAR REFERENCE TO THE INTESTINAL CARRIER.

The discovery of the typhoid bacillus in cases of cholecystitis, cholelithiasis, bone abscesses, &c., long after recovery from the primary infection, was made many years previously to the recognition of the typhoid-carrier. The fact, also, that at post-mortem examinations of persons who have died of typhoid fever, the typhoid bacillus is invariably found in the bile and in the upper portion of the small intestine has been known since the work of Anton and Fütterer (1888) and later of Chiari (1894), and many others. In 1899 Droba (1899) recorded a case of cholelithiasis which was operated on, in which the B. typhosus was isolated from the gall bladder contents as also from the interior of the gall stone. The interval since the primary typhoid infection was 17 years.

Similar finds of the B. typhosus in cases of cholelithiasis at longer or shorter periods after typhoid fever were made by Miller (1898), Brion (1901), Findlay and Buchanan (1906), Simon (1907), Dudgeon (1908), and many other observers.

A definite history of a typhoid infection in these cases may be wanting. Thus Blumenthal (1904) records two cases where there was no previous history pointing to typhoid or any like infection. One of these was a woman of 37 years who did not remember having an attack of typhoid fever. At the operation the gall bladder contained two round stones, the cystic duct and the choledochus were patent, and the typhoid bacillus was recovered from the pus in the gall bladder but not from the gall stones. In this case the fæces and urine were examined and found negative. It must be remembered, however, that a single negative examination of the excreta can lead to no definite conclusion.

Though this person had never had typhoid fever according to her own story, it was ascertained that her husband died of typhoid fever, that her brother and a child of her own (10 months old) suffered from a typhoidal attack in the beginning of January, 1904, while in a house only 100 yards distant there had been five cases of typhoid fever. It seemed therefore highly probable in view of such evident association with typhoid cases, that she may have passed through an ambulant form of fever, culminating in cholelithiasis. Her serum agglutinated the homologous strain in the unusually high dilution of 1 in 2,500. Cushing's (1898) case also presented no history of enteric fever. The Widal reaction was positive, and typhoid bacilli were present in the bile.

The case reported by Finlay and Buchanan in 1906 presents points of great interest in connection with the carrier question, as it is one of the few cases in which examination of the fæces was carried out. A married female, aged 51 years, had suffered from weakness, loss of appetite, and a severe bilious attack two months before seeking advice (August, 1905). In 1880 she had had a febrile attack lasting three weeks, which was thought to be gastric fever. On examination in August, 1905, a large painless tumour was found in the region of the gall bladder. Her temperature reached 103·2° F., and during the first five days while she was under observation the temperature curve suggested that of a declining typhoid fever, but there were none of the classical signs of the disease. Her blood gave a positive Widal reaction (1 in 50 in one hour). A diagnosis of typhoidal cholecystitis was made and was supported by the discovery of typhoid bacilli in the fæces. At the operation on 15th August the gall bladder was found to be greatly distended, and a clear water fluid escaped followed by milky, and finally, purulent material. Drainage was resorted to, and from 19th August to 22nd September typhoid bacilli were constantly present in the discharge. About 30 small stones were washed out at intervals. Bacteriological examination of the discharge on the 22nd and 28th September did not reveal the presence of the typhoid bacillus. On 4th October signs of pleural effusion, breathlessness, and abdominal distension supervened, and death took place on 14th October as the result of cardiac failure. The pleural fluid was sterile. Unfortunately no post-mortem examination was made in this case, but it seems highly probable in the light of very similar cases reported of late, that this woman had been for many years a chronic carrier and had succumbed to an autoinfection. (*Vide* Chapter VIII.)

In a case reported by Dudgeon there was no history of typhoid fever. The patient had served in the Army in India and in 1900 had suffered from a severe attack of colic. No further attack took place until six months before coming to hospital (1908). Jaundice supervened and his blood gave a reaction in a dilution of 1 in 500. An operation was performed and a gall stone was removed which was sterile, but the bile contained typhoid bacilli. The fæces and urine were negative. Three weeks later the bile still showed the B. typhosus.

An acute cholecystitis may however occur at a very early period of convalescence from typhoid fever. The case reported by Gilbert and Girode (1893), is of interest from the fact that a microscopical examination was made of the gall-bladder wall. The patient was a female of 45 years, who developed a purulent cholecystitis in the fifth month of her convalescence from typhoid fever. On operation calculi were found and the typhoid bacillus was present in the pus. Sections of the bladder-wall showed accumulations of small cells in the mucosa, with masses of typhoid bacilli embedded in them.

Hilgermann (1909) gives an account of a woman (aged 29 years) who contracted tpyhoid fever on 23rd August, 1908, and whose temperature fell to normal on 24th September. Shortly afterwards a sudden attack of gall stone colic supervened and in the course of ten days a tumour developed in the gall bladder region. On 4th October, 1908, the gall bladder was extirpated. The fæces on 16th October were negative, but on 20th October, typhoid bacilli were present. Later examinations, however, were negative and she was discharged cured on 20th November. The gall bladder contained 300 c.c. of foul-smelling fluid and a stone of the size of a pea. From the pus the B. typhosus was recovered in pure culture. Histological examination of the gall-bladder wall showed disappearance of the epithelium, and inflammatory infiltrates in the submucosa and muscularis. Typhoid-like bacilli were present only in the subserosa. In Hilgermann's view the bacilli had penetrated from the inside of the gall-bladder through the epithelium into the deeper layers and so to the outer wall. One might therefore readily conceive how, with a chronically inflamed mucosa and the presence of typhoid-nests in the layers of the bladder wall, fresh infections of the gall bladder contents might periodically occur and so in some measure explain the intermittent excretion of bacilli by the fæces. The importance of a case like this lies in the fact that some light is thrown on the pathogenesis of the typhoidal cholecystitis and all such observations are of value at the present time. As we shall see a very similar condition of affairs has been found in fatal cases of enteric fever (Josef Koch (1909)) and experimentally in rabbits (Chiarolanza (1909)).

I have mentioned that the fæces contained typhoid bacilli on the 16th day after operation and thereafter were typhoid free. In connexion with treatment in Chapter X., the value of cholecystectomy as a remedy for the carrier condition will be discussed, but it may be remarked here that, in order to be certain that the patient is bacteriologically typhoid free, a much longer period of observation is necessary. The case, however, was a very early one in typhoid convalescence and, as such, it seems quite possible that a bacteriological cure was affected before chronic changes in the gall bladder and in the neighbouring parts had had time to establish themselves.

A case bearing on this point has been under observation by Theodore Thomson and the author. Mrs. B. had enteric fever in June, 1909, and was discharged from hospital on 2nd July. The last two of the three examinations of her fæces before discharge were positive but the urine was always negative. No further samples could be procured from her after her discharge. She commenced to suffer from gall stone colic in October and was admitted to St. Bartholomew's Hospital on 1st November, 1909. The stools and urine were there examined with negative results on 2nd November. On 19th November she was operated on, when a small gall bladder with thick walls and full of stones was removed. The contents of the gall bladder contained typhoid bacilli but the fæces and urine were negative.

Samples of this woman's stools and urine were examined by me on 7th December, 1909, when the fæces were found to contain typhoid bacilli. She was discharged on 15th December, 1909.

Another examination of the fæces in February, 1910, was negative. It is of great importance that a case like this should be periodically examined with a view to discovering whether a real cure has been effected. It has, however, proved impossible to obtain further samples of the woman's excreta.

Hertz and Adams (1910) have also reported a case in which a purulent cholecystitis accompanied by gall-stones intervened at an early period in convalesence from typhoid fever. The patient (female of 24 years) was admitted to the Brook Fever Hospital on 22nd December, 1909. Her temperature fell to normal on 2nd January, 1910, but a relapse occurred from 14th January to 24th January. On 1st February, 1910, she complained for the first time of pain in the epigastrium and back, accompanied by vomiting. The gall-bladder was found to be enlarged and tender. On 18th February the temperature rose to 99·4°F. and she was sent to Guy's Hospital, where she was operated on the same evening. The gall-bladder was opened and, after about 4 ozs. of colourless clear fluid had escaped, about $\frac{1}{2}$ oz. of pus was evacuated along with 25 facetted cholesterol gall-stones. The B. typhosus was recovered from the pus and from the centres of some of the gall-stones. The gall-bladder was drained and the patient made a good recovery.

Since the discharge of this case from hospital I have had an opportunity of examining her excreta on two occasions, viz., 2nd April, 1910, and 13th May, 1910. On both occasions the fæces were found to contain typhoid bacilli, so that no improvement in the carrier condition can so far be recorded as the result of the operative procedures carried out.

Hitherto I have referred to the finding of the B. typhosus in persons suffering from cholelithiasis who had not previously been regarded as typhoid carriers in the sense in which the term is used in this memoir, but there can be little doubt that many of these cases would have been found to be actively or intermittently excreting the B. typhosus in the fæces had the attention of the bacteriologist or those concerned been directed to this possibility.

Before passing to the discussion of recognised typhoid carriers and their relation to gall-bladder troubles, some mention may be made of the positive Widal findings so often referred to in jaundiced patients before the carrier condition was recognised. According to Kohler (1902), who collected 614 references, which had appeared up to the spring of 1901, concerning the agglutinins in typhoid fever, the prevailing idea then was that the presence of bile elements in the blood serum influenced the agglutinating power of the latter towards the typhoid bacillus. As a matter of fact the bile itself, though it possesses in low dilutions an agglutinating effect on the typhoid bacillus, shows no greater agglutinating power over typhoid bacilli in persons dying of typhoid fever than in persons dying of other diseases.

By injecting into dogs and rabbits the various elements of the bile (taurocholic acid, &c.) or by creating artificial biliary stasis through ligature of the ductus choledochus, Kohler attempted to show that the agglutinating power of the serum towards the B. typhosus is artificially developed. For example, in one experiment before operation the serum gave no agglutination in a dilution of 1 in 10. After operation when bile pigment was present in the urine, the serum gave a slight reaction at 1 in 60, and in another case a slight reaction at 1 in 20. It cannot be admitted, however, that the figures are at all significant and further experiments by Venema (1906) showed that the addition of human bile to serum did not influence its agglutinating power towards B. typhosus or B. paratyphosus.

Carriers and Gall Stone Disease.

Quite a large number of carriers suffer from gall stones, while in others, though symptoms may have been absent, a condition of cholelithiasis has been almost invariably found, whether on examination post mortem or at operations directed towards bacteriological cure of the carrier condition. As early as 1892 Naunyn (1892) had observed that gall stone troubles frequently occurred in persons who had suffered from typhoid fever.

Forster (1908) was the first to direct attention to the association of gall bladder complaint with typhoid carriers. He found that out of a total of 194 convalescent carriers, who continued to carry the bacilli for some weeks after convalescence, 29 per cent. were men, 45 per cent. were women, and 26 per cent. were children. On the other hand out of 173 chronic carriers who continued to excrete the bacilli from 1 up to 30 years after the primary attack, 79 per cent. were women, 17 per cent. were men, and 4 per cent. were children. This striking preponderance of female chronic carriers over male (nearly 5 to 1) suggested a strong analogy to the relative gall stone incidence in the female and male, which is approximately 3 to 1. As a matter of fact 25 (*i.e.*, 14 per cent.) of the 173 chronic carriers referred to presented gall stone symptoms during life, while the remaining 86 per cent. had no such symptoms. This accords well with the known fact that 90 per cent. of all gall stone cases present no symptoms during life. Similar percentages have been obtained by other observers, *e.g.*, Mayer, G. (1910) (*vide* Chapter II.).

There has been considerable discussion as to whether the typhoid bacillus directly excites the formation of the gall stone and even forms a nucleus for it, or whether the stone is preformed and is later penetrated by the typhoid bacillus. The question cannot yet be considered as settled, but the chief arguments on either side may, with advantage be referred to here.

In the first place the typhoid bacillus has been repeatedly isolated from the centre of gall stones in cases of cholelithiasis operated on by the surgeon and in chronic carriers either at operation or at autopsy (*vide* Chapters VIII. and IX.). Individual instances of such findings need not be mentioned in detail, but I may refer here to a carrier case in which the typhoid bacillus has on two occasions been isolated from gall stones passed in the fæces. This case has been under my observation for two years. He is a medical man, and has kept accurate records of his gall stone attacks, which he has kindly placed at my disposal. The primary typhoid infection occurred in November and December, 1896, and was a very severe one with two relapses. Convalescence was established at end of February, 1897, and on August 24th of the same year he had his first attack of biliary colic which was accompanied by slight jaundice and lasted four days. Two further attacks had their commencement on November 10th and December 13th respectively. In 1898 there was only one attack commencing on January 14th and lasting five days. The patient enjoyed a respite from gall stone colic till April, 1902, when five transient paroxysms lasting each half-an-hour occurred on 5th, 11th, 14th, 16th and 20th April. No stone was passed.

On April 21st a severe attack occurred, and on this occasion a mulberry-shaped calculus weighing 4 grains and consisting of bile pigment was found in the stools.

Transient colic attacks were experienced on May 4th and June 23rd followed on July 17th by a severe attack lasting five days and accompanied by slight jaundice.

On July 27th there was a less severe attack lasting two days, and a calculus similar to the first was found.

In 1903 the first attack commenced on March 17th and lasted five days; a calculus was found. On April 11th a similar attack occurred, with passage of a calculus. There were also similar attacks lasting two days and with passage of calculus on May 12th, June 2nd, June 11th, July 3rd, August 18th, and September 15th.

1904. Attacks occurred on July 5th, 7th, and August 18th, with passage of calculi. On October 1st there was a severe attack with renewal of colic on fifth day; two calculi were passed. On November 24th an attack occurred lasting three days; a calculus was passed.

Three years of freedom intervened.

1907. On November 23rd, 24th, 28th and 30th, December 27th and 30th there were transient attacks of colic unaccompanied by passage of stone.

1908. On January 2nd occurred the severest attack yet experienced, requiring the administration of an anæsthetic; a stone was passed. On March 14th biliary colic began and lasted three days. The stone passed on this occasion was submitted to bacteriological examination (at the Lister Institute) and found to contain typhoid bacilli. From that date to the present numerous examinations have been made of his fæces and of occasional gall-stones. On March 24th he suffered from two days' illness; no stone was passed.

1909. On May 5th, 6th and 7th transient attacks occurred, each lasting half-an-hour. Later, on the 7th, a severe attack ensued lasting three days, and accompanied by passage of a stone. On October 3rd prodromal colicky pains began at 11 a.m. and severe colic at 9 p.m., which entailed confinement in bed till 6th October; a calculus was passed.

This last calculus, which was passed on 6th October, 1909, was placed on cotton-wool in a pill box, and was not handed over for bacteriological examination until 3rd November, 1909. It was a brown friable pigment stone of the size of a large pea. Owing to its rough friable and porous character, it was impossible to sterilise the exterior thoroughly. Accordingly it was shaken in a tube of sterile broth, removed, dipped in alcohol and flamed two or three times, and finally ground up in a mortar containing a small quantity of sterile broth. The washings and the stone emulsion were then immediately plated on bile-salt-lactose-agar. Next day the plates contained enormous numbers of typhoid bacilli in pure culture. The strain was agglutinated by an antiserum in dilution of 1 in 7,000, and was lethal for a guinea-pig of 250 grms. weight (intraperitoneal injection) in 0·5 c.c. of a 48-hours broth culture. That the typhoid bacillus would be viable in a gall-stone after a month's sojourn in cotton wool was not anticipated, and in view of the positive result two gall-stones, passed by the same patient on 9th May, 1909, and 29th December, 1907, respectively, were examined in a similar way on 5th November, 1909. Neither, however, contained typhoid bacilli. Kramer (1907) had also observed that typhoid bacilli contained in apparently dried up bile salts retained their vitality for a considerable time.

At the close of Chapter XII. will be found details concerning the bacteriological examination of the excreta of the above case (P.).

A case in many respects similar to the above was reported by Dean (1908). The carrier, who was also a medical practitioner, had passed through a severe attack of enteric fever 29 years previously. Within three months of his illness he had an attack of acute pain in the gall-bladder region, and for more than a year thereafter he suffered from biliary crises at intervals of about three months. Jaundice was occasionally a feature of these attacks. Afterwards, however, the attacks became less frequent (one or two per annum), and they have never led to any interference with his medical work. His fæces showed on examination (in spring of 1908) enormous numbers of typhoid bacilli, but the urine was negative. His serum gave a negative Widal reaction. Since 1908 numerous examinations of his fæces have been made with, as a rule, positive results. Neither of the above two cases is known to have caused infection in other persons.

Bacmeister (1907) reported the bacteriological examination of a series of 20 gall-stones from various cases. Of these 16 were sterile and four contained bacilli in the centre, viz., B. coli (two cases) and B. typhosus (two cases). One of these latter was a female who died of typhoid fever in the fourth week of the disease. There was no evidence in this case of a previous attack of typhoid fever, and he considered it very unlikely that in three weeks the typhoid bacillus could cause the formation of a hard stone of the size of a cherry, although Mignot (1898), Droba (1899), Naunyn (1892), believed such rapid formation possible. Hertz and Adams (1910) calculated that, in their case reported above, the gall-stones must have taken less than 68 days—probably not more than 50 days to form. The evidence they assign, however, is not indisputable as, although there was no history of an earlier typhoid infection, the authors explicitly state that the patient had occasionally suffered from a dull aching epigastric pain since the birth of her only child three years ago. In Bacmeister's view stones found in recent cases of cholecystitis were as a rule sterile, while organisms were only to be found in old stones into which they had penetrated. The opposite view has been held by other observers, *e.g.*, Cushing, viz., that only recently formed stones contain bacilli. Gilbert and Fournier (1896) had previously experimented with gall-stones and broth cultures of B. coli and found that the bacilli wandered in when the stones were of cholesterin, but not in the case of other stones. Their experiments were repeated by Bacmeister, who placed gall-stones in both broth and bile cultures of B. typhosus. Most of the stones remained sterile, but in two cases (after eight days and 24 days respectively) the B. typhosus was recovered from the centre of the stone. One of these stones was a mulberry cholesterin stone of the size of a hazel-nut while the other was a facetted stone of the size of a cherry. They had been obtained from two women of 38 years and 82 years of age respectively. It may be noted that no mention is made of the history of these women or of the interval between the experiment and the obtaining of the stones. The possibility is not excluded that these cases had been carriers, and the typhoid bacilli may still have been viable in the stone as in the case referred to above.

The fact that bacilli can penetrate into the interior of gall-stones is a point of great importance, but it by no means follows that in the genesis of gall-stones micro-organisms play an entirely passive role. The experiments of Gérard (1905), Kramer (1907), Bacmeister (1908) and others go to show that embryonic gall-stone formation may be observed in test-tubes containing bile cultures of B. typhosus, B. coli, and other micro-organisms. The last-named observer found that B. coli and B. typhosus, B. proteus, and especially B. pyocyaneus could cause a precipitation of cholesterin from filtered bile. True concretions were also found after prolonged growth on this medium. Their ability to produce such precipitates was much enhanced by employing unfiltered bile containing epithelial elements. Similar precipitates of cholesterin also occurred in sterile filtered bile in which no micro-organisms had grown, so that in the genesis of the pure cholesterin stone, at least, stasis alone can act as the exciting factor. The presence of epithelial elements or bacteria in the test-tube experiments, merely accelerated and intensified this precipitation of cholesterin. For the genesis of the mixed cholesterin-chalk stone, however, they believed that a chronic inflammatory condition of the bladder wall was essential. Exner and Heyrovsky (1908) performed similar experiments with ox bile and human bile and also with broth to which the bile salts were added. They found that some micro-organisms, especially those of the typhoid-group, could readily break down the bile salts (sodium glycocholate and sodium taurocholate), thus causing a precipitate of cholesterin. In this reaction they believed that the soaps and fatty acids of the bile played no part. They found, indeed, that the bile contained only a very small proportion of true fatty acids.

In the light of these experimental results there would seem to be no doubt that the typhoid bacillus may play a part in the formation of gall-stones, but at present we are unable to say how far this formation is contributed to by other factors.

Our knowledge of the relationships of B. typhosus to the gall-bladder has been considerably enhanced by animal experimentation. So long ago as 1899, Blachstein (1899) and Welch (1899) in America demonstrated that in rabbits which had been inoculated intravenously by typhoid bacilli the gall bladder might continue to harbour these organisms for a very long period, even up to 109 days.

Forster and Kayser (1905), the former of whom, as I have already noted, put forward the view that the gall bladder was most probably the seat of vegetation of the typhoid bacillus in carriers, repeated the experiments of Blachstein and Welch with a view to reproducing the carrier-state in the rabbit. They also found that after large doses given intravenously the bile might still be infective six weeks later. Typhoid bacilli could also be recovered in some cases from the upper intestine. Further, they made the important observation that in cases in which the bile was sterile the typhoid bacillus could occasionally be isolated from the gall bladder mucosa. Similarly in man at a very early stage in the disease the bacilli would reach the gall bladder and there set up definite lesions which might or might not be accompanied by distinctive clinical symptoms. According to Forster and Kayser the bacilli would reach the liver by the blood, be excreted by the bile and so reach the gall bladder.

In the same year Doerr (1905) recorded the results of experiments undertaken to test whether the typhoid bacilli reached the gall bladder by the downward route from the liver through the blood, or by the upward route from the intestine. They showed that bacilli appeared in the gall bladder as early as eight hours after intravenous inoculation. When, however, the hepatic duct was ligatured the bile invariably remained sterile. Also when the cystic duct was ligatured no bacilli could be demonstrated in the bile, thus proving that the bacilli travelled by the bile and not by the blood vessels of the gall bladder itself. These latter experimental results dealing with ligature of the cystic duct have recently been called in question by Koch, J. (1909), and Chiarolanza (1909). The former (at the instigation of Frosch, who believed that not only the gall bladder, but also the biliary tracts and perhaps the liver itself were seats of vegetation of the typhoid bacillus in carriers) investigated the histology of the gall bladder mucosa in a fatal case of typhoid fever. The patient had died of heart failure in the third week of the disease.

The organ contained cloudy, slimy, green bile, and its walls were much thicker than normal. No stones were present. Typhoid bacilli were recovered from the gall bladder wall, the larger biliary tracts, the mesenteric glands, renal pelvis, and small intestine, but not from the heart blood. On microscopical examination the mucosa of the gall bladder was very much corrugated and papillated, and, near the extremities of the papillæ, typhoid nests were found with necrotic areas in their vicinity. The superficial epithelium had completely disappeared and there was a marked inflammatory proliferation of the sub-mucosal folds. A conspicuous feature was the close relationship of these typhoid nests to the minute end-capillaries of the sub-mucosal papillæ, suggesting that the bacilli had reached this situation solely by way of the blood vessels. No organisms were demonstrable microscopically in the lumen. The experiments of Chiarolanza entirely supported this view. Even within two hours of intravenous injection, typhoid bacilli were recovered from the gall bladder wall. Also, contrary to Doerr's results, bacilli were got from the gall bladder when the cystic duct was ligatured. The histology of the rabbit's gall bladder was essentially similar to that of the human case above referred to. The walls were thickened and infiltrated and the sub-mucosa very cellular with papillary proliferations containing typhoid nests at their extremities. Sections of the bile tracts and the intestine showed parallel changes, and there was a marked small-cell proliferation in the portal areas of the liver itself. It would appear, therefore, that the main, if not the only route, of the typhoid bacilli to the gall bladder is by the vessels of that organ itself. In any case this is the chief route to be considered in drawing deductions as to the possible value of surgical interference with the gall bladder as a remedy for the carrier-state. The bacilli vegetate in these sub-mucosal nests and not in the bile itself. The latter passively receives accessions of typhoid bacilli from ruptured nests either in the bladder mucosa or in the biliary tracts. I shall further discuss the bearing of these facts in a later chapter (*vide* Chapter IX.).

I shall conclude this section with certain general considerations which have been put forward in explanation of the carrier state. In the first place with regard to the preponderance of cholelithiasis in the female, various reasons have been assigned, such as the wearing of tight corsets, frequent pregnancies, &c., causing portal stasis.

Similar factors may, however, according to Forster, play a part in rendering males liable to carry the bacillus indefinitely after the primary attack. Thus Forster (1908) mentions the case of a man who contracted typhoid fever two years after an operation for appendicitis. After his recovery his wife took typhoid fever and the husband was then found to be a carrier.

Owing to the appendix operation and ensuing inflammation anatomical changes had taken place in the peritoneum, thus altering the circulatory conditions and probably predisposing to the carrier state. This explanation by Forster may have some foundation in fact, but further data must be awaited before one can draw any definite conclusions on this point. One may note, however, that Lentz (1905) had already suggested that the greater liability of women to become carriers probably bore some relation to the circulatory disturbances incidental to pregnancies and to over-exertion in household duties.

An interesting view lately put forward by Prigge (1909) in order to explain the higher proportion of women carriers may also be noted here. Whereas, according to Prigge, the incidence of enteric fever on females reaches a maximum between the ages of 15 and 20 years, the probability that a woman will become a chronic carrier increases gradually from the 15th to the 45th year. This fact suggested to him the possibility that predisposition to the carrier state might be induced by the menstrual and puerperal blood losses. There is some evidence that these physiological crises are accompanied by a diminution of the alexin-content of the blood serum, which would render the subject for the time being more vulnerable to an infecting micro-organism. In support of this view it is alleged that enteric fever (often mistakenly diagnosed as puerperal fever) is of frequent occurrence at childbed. If to the general blood changes at these seasons there be added the pressure caused by a gravid uterus on the biliary system, there would, it is maintained, be a sufficiency of factors predisposing to the carrier state. As Bacmeister showed in his in vitro experiments, stasis fluids accelerate the precipitation of cholesterin by bacteria. If therefore a woman who has previously had an attack of cholecystitis contracts enteric fever, the chances of her remaining a carrier after her attack would be intensified.

Fornet (1909) has recently formulated the view that the carrier state is to be regarded as a consequence of established immunity to the typhoid bacillus, and that, in fact, the bacilli discharged by the carrier may not necessarily be descended from those which caused the primary infection. A re-infection takes place which, according to Fornet, is not accompanied by any clinical symptoms. The region attacked by the bacilli in this re-invasion, would have acquired, in consequence of the primary attack, such a tolerance to the micro-organism as to render its saprophytic existence possible.

This view was based by Fornet on the following considerations:—

Klinger had found for the year 1907 of the typhoid campaign a carrier output of only ·9 per cent., whereas most individual observers have obtained a much higher percentage (*see* Chapter II.). Fornet thinks, therefore, that persons may not become carriers as the result of the primary infection, but only after a second symptomless infection. The carrier statistics for the year 1908 (compiled by Fornet) are adduced in support of his view. Thus, in that year, 91 carriers (temporary and chronic) were discovered in the course of the typhoid investigations, and, as Klinger had already noted in his series (Chapter II.), the group of chronic carriers was almost exclusively made up of adults, whereas among the temporary carriers children formed a large contingent (30 per cent.). Fornet argues that, as the adult has probably had more opportunities of having passed through an attack of typhoid fever than the child, the prospect of his becoming a carrier as the result of a second infection would be correspondingly greater. Accordingly, if his view that the carrier state is the culmination of a symptomless re-infection be a correct one, he would expect to find among the chronic carriers of the year 1908 a greater proportion of adults who had already had typhoid than of adults who had not so far suffered from the disease. As a matter of fact 59 per cent. of the chronic carriers had already had typhoid, while 41 per cent. had not yet had the infection. Among the temporary carriers, on the other hand, 74 per cent. had not yet had the disease, while 26 per cent. had recovered from a primary attack.

It does not appear to me valid to employ these statistics in this way, in view of the difficulty of determining whether a person who happens to be a temporary carrier has really passed through a typhoid infection or not. While recognising the important distinctions that separate the two groups of chronic and temporary carriers particularly in the matter of age and sex, it would be unjustifiable in the present state of our knowledge to lay stress on the numerical distinctions between the carriers in each group who have had a previous infection and those who have not. Indeed, from Klinger's collected statistics up to 1907 (*see* Chapter II.) it is seen that 80 per cent. of the carriers had had a primary infection, while 20 per cent. had not. Of the temporary carriers 43·6 per cent. had had a primary infection, while

56·4 per cent. had not. Thus the relative percentages in this latter group differ considerably from those of Fornet for the year 1908.

Finally, in view of the gradual accumulation of cases which have been traced bacteriologically from the convalescent stage onwards to the chronic carrier state, such a theory as that of Fornet would seem to me at present uncalled for. It is undoubtedly, however, an interesting speculation, and it remains to be seen what amount of support is lent to it by the results of experimentation on the saprophytic growth of pathogenic bacteria in the immune organism.

CHAPTER VIII.

PHYSICAL DISABILITIES OF THE CARRIER STATE, AUTO-INFECTION, &C.

The question is frequently asked whether the typhoid carrier suffers in bodily health as the result of the carrier state, or whether *ceteris paribus* he or she remains a normal healthy individual. In general it may be said that the carrier who has no definite gall bladder symptoms is little if at all inconvenienced in bodily health by the fact that the typhoid bacillus has acquired a permanent habitat in some portion or portions of his anatomy. Occasionally headaches are complained of, particularly by urinary carriers, and measures taken to arrest the typhuria have reacted favourably on the general well-being of these cases.

Mayer, G. (1910), however, has laid stress on the fact that certain carriers of the intermittent type have been observed to suffer from periodic intestinal disturbances after the primary infection. He states that these are specially likely to occur at certain seasons, *e.g.*, in midsummer, when winter merges into spring, autumn into winter, and that at such seasons it is advisable to exercise strict bacteriological supervision over declared carriers. Also it must be admitted that in persons of nervous temperament who are aware of their condition there is good evidence that the knowledge of their having caused disease in other persons, the consequent anxiety and the necessarily harassing precautions to be taken with regard to disinfection, &c., develops a form of neurosis which may react injuriously on the general bodily health. Such cases have not infrequently been met with, and the fact that the carrying-state may have this unfortunate and serious issue calls for the greatest tact on the part of those whose duty it is to exercise supervision over carriers.

In this section I propose to discuss mainly those cases in which a recognised carrier suddenly develops a train of symptoms pointing to a general infection by the bacillus he or she is carrying. Only a few examples have so far been reported in which an auto-infection has been indicated, but they are of such importance for an understanding of the carrier-state, that they will be referred to in some detail.

The first of such cases was recorded by Levy and Kayser in 1907. The patient (female, aged 35 years) was an inmate of the Hordt Asylum, an institution in which 13 other carriers were discovered at various times (1904–5). She was admitted to the Asylum as an epileptic on 25th March, 1903. During April and May of the same year she suffered from a remittent fever, which was diagnosed as typhoid fever although no Widal or other bacteriological test was made. Two years later when carriers were being sought for to explain the typhoid epidemics in the asylum, this woman was found to be a carrier (16th October, 1905). Further examinations of her stools on 24th November, 1905, 25th January, 1906, 6th February, 1906, 2nd March, 9th April, 27th April, 28th May, 11th June, and 29th June, 1906, were positive, while the urine remained negative. On 17th October, 1905, she had been isolated along with the other carriers and a year later (8th October, 1906) she became ill with pain in the stomach region and constipation. On 12th October she was put to bed and death took place on the 18th, following a febrile attack associated with severe pulmonary symptoms. The autopsy (19th October) revealed hypostatic pneumonia of the right lower lobe and a slightly enlarged spleen. The brain appeared normal. The gall bladder contained a cholesterin stone of the size of a bean. Its peripheral layers were sterile but from the centre the B. typhosus was recovered. This bacillus was also isolated from the liver-blood, the spleen, the bile and gall bladder wall. Though the post-mortem examination was performed 19 hours after death, the bacteriological results, particularly the finding of typhoid bacilli in the spleen and liver-blood and the absence of other micro-organisms led to the conclusion that the carrier had succumbed to a general infection by her own bacillus. The ill-defined pains in the stomach region on 8th October, 1906, might very well fit in with gall-bladder trouble, as it is well known (Naunyn, 1892, Riedel, 1905) that pain arising from disturbance of the latter organ may be referred to the stomach region. The discovery of the bacillus in the centre of the stone was naturally held by Levy and Kayser to give strong support to Forster's view that the gall bladder is the seat of vegetation of the typhoid bacillus in carriers.

The second case reported by Grimme (1907) as one of auto-infection will be referred to here, though the actual bacteriological proof that the woman had been a chronic carrier was wanting. The patient (aged 52 years) was also an inmate of an asylum. Suddenly when at work she developed epileptiform convulsions and became unconscious. Her temperature was 38 °C. and there was slight splenic enlargement. Vomiting occurred while the patient was being examined. On the second day, jaundice developed and the liver and spleen increased in size. There was no rash. On the third day a Widal test was positive (1 in 800) and the B. typhosus was got from the blood and fæces. Death occurred on the twelfth day. At the autopsy a cancer of the gall bladder was found which had led to the closure of the ducts and so to jaundice. One gall stone was present but the typhoid bacillus was not recovered from it. The spleen, bile and peritoneal exudate, however, contained typhoid bacilli. Peyer's patches were not swollen but appeared atrophic with bluish pigmented patches in them like the remains of some previous pathological condition. That the patient had had typhoid fever previously was rendered probable from the fact that in 1904 when outside the institution she had suffered from a febrile attack with diarrhœa, slight splenomegaly and a doubtful rash.

A very similar case, affecting also a female lunatic (64 years of age), is reported by Kamm (1909). This woman in November, 1901, passed through a typical attack of enteric fever. On 12th March, 1906, in the course of a systematic examination of the female inmates, she was discovered to be a carrier. Positive results were obtained from her stools on 8th and 20th March, 9th and 27th April, 25th May, 2nd, 12th and 20th June, 28th July, 8th and 14th August, 19th October, and 9th December, 1906, but the urine was invariably free from typhoid bacilli. In spite of all therapeutic efforts to free the patient of her typhoid bacilli, 72 out of the 85 examinations of her stools during 1907 were positive. In February-March, 1908, pulmonary troubles pointing to phthisis intervened and tubercle bacilli were found in the sputum. Death occurred on 16th March, 1908, after an attack of cerebral apoplexy lasting four days. The autopsy revealed carcinoma of the gall bladder encroaching on the liver, cholelithiasis, secondary growths in liver, pleuræ, lungs, and retroperitoneal glands. The ductus choledochus and ductus hepaticus were normal. The cystic duct was thickened and its lumen so narrowed that a sound could not be passed through the opening into the choledochus. The gall bladder was much dilated and contained 195 polyhedral stones of which the largest weighed 18 grammes. The spleen was enlarged but not the mesenteric glands. From the gall bladder, the biliary tracts, the interior of the large stone, the right and left lobes of the liver, the lungs, bone marrow, and spleen and all portions of the intestinal tract, the typhoid bacillus was recovered. It was not present, however, in the heart blood, the small stones, or the cervical mesenteric and retroperitoneal glands. How far this general infection by the typhoid bacillus had contributed to the patient's death it was impossible to say. The author refused to consider that the typhoid bacillus had merely invaded a weakened organism for the reason that, in another case observed by him (*vide* Chapter IX.), where the bodily enfeeblement was equally great, there was no general distribution of the typhoid bacilli. With the view of throwing some light on the matter Kamm made an interesting experiment which may be quoted here. Under an anæsthetic he introduced a piece of pumice stone (of the size of a pea) saturated with a broth culture of the typhoid bacillus into the gall bladder of a rabbit (16th December, 1907). The bladder was then sewn up and the animal quickly recovered. Repeated examination of the blood after the operation showed that no invasion of the blood stream by the typhoid bacillus had taken place during the operative procedures. Judging that the animal would still be carrying the bacillus at a period of three weeks after the operation, he selected uranium nitrate as a substance calculated on injection to produce an extremely weakening effect upon the animal. On the 20th, 21st, 22nd and 23rd days, therefore, after the operation, 1 c.c. of a 5 per cent. solution of this substance was injected. Extreme emaciation resulted, followed by death five days after the commencement of this treatment. At the autopsy the kidneys showed parenchymatous nephritis, but the typhoid bacillus was not recovered from the blood, spleen or kidneys. It was, however, isolated from the bile, the pumice stone, the duodenum, ileum and mesenteric glands. Thus the uranium nitrate did not lead to a general invasion by the typhoid bacillus and the problem has to be left undecided. Further experimental work on these lines is desirable as there has been a tendency in recent years to attach a perhaps unwarrantable degree of importance to those bacillary invasions which have been found to occur in mentally enfeebled persons.

The instances I have mentioned so far have all been fatal cases in which the auto-infection was verified by extensive bacteriological examinations made after death. The literature of this subject also contains not a few examples of typhoid relapses in typhoid carriers which did not lead to a fatal issue.

Davies and Walker Hall (1908) record that Mrs. H., the Brentry carrier discovered by them, developed a febrile attack on 21st May, 1908, which lasted about three weeks. No gall-stones were found in the fæces, and there were no clinical signs of biliary obstruction. It seems doubtful whether this illness was a genuine auto-infection (enteritis or cholecystitis). The agglutinins remained stationary and typhoid bacilli could not be isolated from the blood.

Mayer (1910), in the course of his typhoid investigations in the Bavarian Pfalz, gives a series of such cases which are of considerable interest, inasmuch as the carriers affected were generally intermittent carriers. After the primary attack of typhoid fever the bacilli could be demonstrated only at intervals in the fæces. With the onset of the relapse the excretion of typhoid bacilli became again a prominent feature. There was evidence too, that the Widal reaction, which may have been negative after the primary attack (though the person was still excreting the bacilli intermittently), became positive during the relapse to disappear again after recovery. In Niven's case, referred to in Chapter III., there had been a similar variation in the Widal reaction ; but there was little or no increase in the agglutinating power of the serum, either during or after the above-mentioned illness of Mrs. H. the Brentry carrier.

An interesting case of acute abdominal infection in a typhoid carrier has recently been reported by Gardner (1910). The patient (male, aged 56 years) had an attack of typhoid fever in 1906, when he was in bed for 10 weeks. In 1900 he had suffered from peripheral neuritis which was attributed to the presence of arsenic or lead in the beer "of which he drank a good deal at that time." The illness reported by Dr. Gardner commenced on 26th January, 1910, with pain in the abdomen and nausea, and lasted about a fortnight, the symptoms throughout being those of an acute cholecystitis. There was only very slight icterus, but the fæces after a calomel purge were "semi-solid homogeneous slaty-grey and very offensive." No bacteriological examination of the stools was made until the occurrence of two cases of typhoid fever in the patient's household shortly after his recovery, suggested the possibility that this man might be a typhoid carrier. This proved to be the case. His serum gave a positive Widal reaction, and his fæces were examined by me on 4th April, 1910 with positive results.

Further examinations:—21st April, 1910, positive. 7th May, 1910, negative. 19th May, 1910, negative. The urine was always negative.

Mention must also be made of the possibility of infection of the foetus in pregnant carriers either as the result of a blood invasion by the bacillus consequent on the disturbances during pregnancy and child-bed, or more probably in consequence of an actual cholecystitis during child-bed, as in a case reported by Mayer (1910), where the child was born with typhoid fever. In a later chapter (Chapter XII.) I shall refer to the successful isolation of the typhoid bacillus from the blood of the umbilical cord, at the confinement of a carrier who has been under bacteriological observation for a considerable time. The child, so far as I am aware, was quite healthy at birth.

CHAPTER IX.

Treatment of Typhoid-Carriers.

Intestinal Cases.

Forster (1908) has stated that if we can cure carriers we can get rid of typhoid fever, and, indeed, it may be that only time and further experience are necessary to establish the truth of this dictum. Unfortunately, it must be admitted that hitherto, no certain method has been devised of rendering carriers typhoid-free. That cases have occurred in which the excretion of typhoid bacilli has ceased spontaneously is undoubtedly true, the cessation, perhaps, coinciding with a healing of the gall-bladder trouble. It has even been noticed, as in a case reported by Forster (1908), that the excretion stopped after an acute attack of gall-stone colic which was the first that this female carrier had experienced. We cannot exercise too much caution in accepting these spontaneous cures, as it has been repeatedly demonstrated that an intermittent period may extend over many months, and further it must be remembered that our bacteriological methods are not yet sufficiently delicate to detect minimal quantities of typhoid bacilli in excreta. As an illustration of the necessity for caution in interpreting these so-called cures in temporary carriers, I may quote a personal experience. Mrs. C. had enteric fever in July–August, 1908, and her temperature had fallen to normal on the 24th of the latter month. In late convalescence she suffered from gall-stone complaint. On

30th October, her fæces were examined and found to contain typhoid bacilli. A positive result was also obtained on 8th December, 1908, but further examinations in January, March, April, May, July and August of 1909, were negative and accordingly this case was considered to have belonged to the category of temporary carriers. In January, 1910, however, another examination of her stools revealed enormous numbers of typhoid bacilli, so that the woman is to be regarded in future as a chronic carrier (*see* Table at close of Chapter XII.).

With a view to determining whether spontaneous cures occurred, Prigge (1910) made monthly examinations in the course of the last three years of his chronic and transitory carriers, with the result that of 28 chronic female carriers that had excreted typhoid bacilli over a year, one only could be struck off the list. However, in a foot-note to his paper he mentions that this woman was again excreting typhoid bacilli in October, 1909, after three years intermittence.

In other cases he noted intermittent periods extending from one to two-and-a-half years.

The drug treatment of intestinal carriers has been entirely ineffective in ridding these persons of their typhoid bacilli. Salol, beta-naphthol, calomel, bile and its salts, turpentine oil, sodium bicarbonate in large doses, chloroform, &c., have been employed with no effect or only a temporary one on the excretion of the bacilli. Recently, Hilgermann (1909–*b*) has reported what he regards as a conspicuous success in at least one chronic carrier, after prolonged treatment with sodium salicylate. The carrier was a female lunatic of 71 years. During the period from 30th September, 1905, to 31st October, 1907, her stools had been examined 19 times (*i.e.*, on an average ·73 times a month) and on 18 of these occasions they had been positive. Treatment by sodium salicylate was begun on 11th November, 1907. From this date to 11th December, 1907 she received 3 grams per day (or 45 grs. in 15 gr. doses t.d.s.).

From 12th December, 1907, to 11th February, 1908, 4 gms. per day.
„ 12th February, 1908, to 18th March, 1908, 5 gms. „
„ 4th November, 1908, to 11th November, 1908, 6 gms. „
„ 11th November, 1908, to 20th November, 1908, 5 gms. „

The result was that during the period 16th November, 1907, to 5th May, 1908, the stools were positive in 10 out of 11 examinations (32 up to 6th July, 1909), or three times monthly, were negative.

The intermittence is certainly a striking one, but without more prolonged observation one cannot regard this case as cured. Two other female carriers received similar intensive treatment with sodium salicylate without showing any marked improvement. It may be noted that 5 gms. per day (or 15 grs. five times a day) were well tolerated, but higher doses caused general disturbances.

So far as the results of drug treatment are concerned all that one can conclude at present is that internal antiseptics may, if pushed to their physiological limits, offer some slight prospect of success. Our knowledge of the pathology of the carrier state makes it difficult to conceive that any treatment other than an intensive one should exercise the slightest effect on indolent typhoidal lesions in the gall-bladder and bile tracts.

The administration of milk soured with lactic acid bacilli has been vaunted by some observers as affording an apparently permanent cure. Thus Liefmann (1909) gave Yoghurt in quantities of one-third to two-thirds litre daily to two female asylum carriers, and observed that one week after the commencement of the treatment the stools in both cases were negative. After seven weeks the stools of one of the cases were again positive, but later examinations were negative. The last examination (there were only eight in all in each case) was made at the end of the eleventh week. Evidence derived from such a short period of observation proves nothing, not even that the temporary cessation was attributable to the treatment employed.

Three asylum carriers (Thomson and Ledingham (1910)), who have been under bacteriological supervision since the latter part of 1907, received sour milk (half-a-pint three times a day) daily during the period February–December, 1909, without any appreciable effect on the excretion of typhoid bacilli in the fæces during the administration of the milk.

Captain Fawcus, of the Army Medical Department (1909–1910), has recently reported fully on the treatment of army carriers by lactic acid bacilli. Two carriers received a course of Bulgarian bacilli grown in diluted malt extract, commencing with a dose of 25 cub. cent. of the culture. No improvement whatever took place in one case except a temporary diminution in the numbers of bacilli excreted, after administration of the doses. The second case, however, reacted more favourably to the treatment. The course was commenced on 8th March with a dose of 25 c.c., and was continued till 28th May when 250 c.c. were being given daily. On 29th April the fæces were apparently typhoid-free, and from that date they remained so. The man was discharged to duty on 23rd July having been free from typhoid bacilli for three months. Further data with regard to this case will be awaited with interest.

At Netley, Major Cummins (1910) had no success with this method of treating intestinal carriers.

I now come to consider those attempts which have been made to abolish the carrier-state by surgical interference with the gall-bladder. Naturally when the view was put forward that the gall-bladder is the main seat of vegetation of the typhoid bacillus in intestinal carriers, operative interference directed towards improvement of the gall-bladder condition, suggested itself as a possible remedy for the carrier-state. The findings at such operations and at post-mortem examinations, which in some instances subsequently took place, are of much interest. I therefore propose to refer in some detail to all those cases which have been treated surgically.

Dehler (1907) operated on two female asylum carriers who had given rise to four cases of typhoid fever, including one fatal case.

Case 1 (aged 46 years) presented no history of having passed through an attack of enteric fever. Her fæces were regularly examined every three weeks, and, in 37 out of the 39 examinations made, typhoid bacilli were present. They were not found in the urine or blood. Her blood serum gave an agglutination reaction of 1 in 50, and often 1 in 100. There were no symptoms pointing to gall-bladder trouble.

On August 20th, 1906, an operation was performed. The gall-bladder was released from surrounding adhesions and opened. Two cherry-sized stones were removed, one of which had dilated the cystic duct. Typhoid bacilli were present in the bile but not in the stones. Drainage was then carried out through a tube led into the dilated cystic duct. The bile regularly contained typhoid bacilli up to the 21st day. It then remained negative until 11th October, 1906, when it was again positive. After the cystic duct could no longer be opened owning to cicatrical formation in the depth, the secretion from the fistula ceased to contain B. typhosus. The fistula cicatrised completely about the middle of December.

Three days before the operation, typhoid bacilli were found in the fæces. Subsequent to the operation 26 samples of fæces were examined in the period, 24th August–17th October, and 50 samples between 17th October and 19th February, 1907, and all were negative. The urine remained typhoid free, and the agglutinins also remained at the same titre as before the operation. Dehler noted that so long as the bile flowed freely and speedily from the fistula, the B. typhosus was scarce and was indeed for four weeks not demonstrable in the bile, but that, as the fistula narrowed and the bile flowed more slowly, the typhoid bacilli got time to propagate in the bile and were therefore very numerous.

Later still, as the fistula closed and the gall-bladder cicatrised, the bacilli disappeared as there was no further opportunity of vegetating in stagnant bile. The fact that the fæces remained negative suggested that any bacilli carried down directly from the bile paths into the intestine were overgrown by other bacteria. Also it was considered not improbable that the discharge of the bacilli from individual portions of the liver and their bile tracts would gradually cease completely. To hasten this action, cholagogues, such as calomel, &c., were given for two months.

In a later communication (22nd October, 1907) Dehler (1907) notes with reference to the above case, that on three occasions out of 176, typhoid bacilli were got in small numbers in the fæces, but believed that these few positive results might be explained by accidental mixing up of samples from other carriers. Up to February, 1907 the agglutination titre remained at 1 in 100, in March and April it fell to 1 in 50, and during May, June, and July it was quite negative. The patient remained in excellent health and the symptoms of intestinal catarrh, from which she had previously suffered, disappeared.

Case 2 (aged 45 years) had no record of typhoid fever, though she had an illness 19 years previously which might possibly have been an anomalous attack of this disease. In July, 1904, her serum had given a positive Widal reactiion, but it was not till 3rd January, 1905, that typhoid bacilli were demonstrated in her fæces. During 1905, nine samples of fæces out of 16 were positive, and during 1906, five out of 10. The urine was always negative. From

January to 9th April, 1907, 16 samples were positive out of 20, and two urine samples were positive out of 14. No gall-bladder symptoms were present during the last three years. Internal remedies had been tried with no result. On 10th April, 1907, the gall-bladder was opened and found to contain several small stones which were sterile. The bile was clear and contained numerous Bac. coli and moderately numerous typhoid bacilli. Drainage was carried out as in the last case.

The bile that drained away during the first few days after operation contained typhoid bacilli, and these disappeared as the bile flow diminished. After the 9th day from the date of the operation they were found only on two occasions in the fæces, viz., 25th May and 3rd July, 1907 (out of 30 samples). Of these 30 samples of fæces, 28 were negative ; the blood and urine remained always negative. The agglutination titre on 31st July, 1907, was positive in 1 in 100, but on 16th August, 1907, a negative reaction was obtained.

In the foregoing instances the surgical interference was confined to drainage of the gall-bladder ; in those that follow removal of the gall-bladder was resorted to.

Grimme (1908) reported that he had had the operation of cholecystectomy performed in a female asylum carrier in a Göttingen asylum. She had never shown symptoms pointing to implication of the gall-bladder.

At the operation (performed by Braun) numerous small stones of the size of peas (30–40) were found in the gall-bladder. No inflammatory changes were present, but the bile and also the interior of the stones contained typhoid bacilli. The gall-bladder wall did not contain them. Fifteen days after operation the B. typhosus was found in the fæces, but not at a later period. The period of observation, however, was only 55 days, as the patient had to be discharged from the asylum.

Loele (1909) reports the next case, also a female lunatic, aged 51 years. This woman had typhoid fever during October and November, 1908, but during convalescence no typhoid bacilli were demonstrated in the fæces.

At Christmas, 1908, she had an attack of gallstone colic with jaundice, and the fæces were now positive. There was a palpable tumour in the gall-bladder region. Her serum gave a reaction of 1 to 2,000, but the blood and urine were sterile. An operation was carried out on 16th February, 1909. The gall-bladder was very adherent and difficult to remove. Between it and the stomach was an abscess containing a stone. During the operation the duodenum was perforated. The cystic duct was quite stenosed. The hepatic duct was drained and the laparotomy wound closed except for the drainage tube and plug.

On 17th February a large flow of bile occurred, and on following days the contents of stomach and duodenum appeared with the bile. Death took place on 20th February. The gall-bladder contained pus and 21 stones of the size of hazel-nuts. The bacteriological results in this case are of great interest.

The gall-bladder pus contained typhoid bacilli, as also did the gall-bladder wall. On the first day after operation the bile contained numerous typhoid bacilli. On the third day the fæces also contained a few typhoid bacilli. The gall-stones were found to be sterile internally.

At the autopsy cicatrices of typhoid ulcers were found in the small and large intestine and bacteriological examination revealed typhoid bacilli in the following situations: small intestine (upper, middle and lower portions), transverse colon, liver, spleen, and heart blood. The urine was negative.

Loele concludes that the typhoid bacilli in the fæces did not come from the gall-bladder for the following reasons. The gall-bladder was quite closed off by stenosis of the cystic duct, and contained pus but no bile. The bacilli may have grown in the dilated choledochus or in the intestine itself, either after wandering in from the bile tracts or after getting accustomed to the small intestine. The smallest bile tracts in the liver were negative.

He does not think that removal of the gall-bladder in this case would have done the slightest good.

It is interesting to note that he found no typhoid nests microscopically in the bladder wall. He thinks the stones were certainly present before the typhoid attack, though they gave rise to no symptoms. The fresh attack had caused new inflammatory changes and empyema. His conclusion, therefore, was that cholecystectomy can be of no use if the seat of vegetation is in the bile tracts or in diverticula of the upper intestine, and that in no circumstances is the operation justifiable in carriers without positive palpatory evidence before operation.

We have seen that in all the carrier cases mentioned above, which have been operated upon or have come to autopsy, cholelithiasis has been invariably present. The following case, reported by Kamm (1909), which succumbed to pneumonia before the contemplated operative procedures could be undertaken, is of interest as showing that gall-stone formation may be absent in chronic carriers. The patient was a female lunatic (61 years old) who passed through an attack of enteric fever in March, 1906. Typhoid bacilli were present in her stools during the attack and were also demonstrated on the following dates: 9th April, 7th May, 28th May, 2nd June, 11th June, 28th July, 11th September, and 7th November, 1906. Isolation had an unfavourable effect on this woman, who suffered from chronic mania. In 1907 typhoid bacilli were found in the fæces (but not in the urine) on the following occasions, viz., 18th January, 15th March, 10th June and 26th July. The female warder who attended to this patient contracted enteric fever on 18th October, 1907. The operation for removal of the gall-bladder was arranged for 30th October, but had to be deferred owing to the onset of croupous pneumonia. Death took place on 20th November, 1907. At the autopsy, in addition to the pulmonary lesion (croupous pneumonia of right lower lobe) there was marked interacinar cirrhosis of the liver, but the gall-bladder showed no inflammatory changes and contained no stones.

Typhoid bacilli were recovered from various parts of the liver, the gall-bladder, and the duodenum, but not from any other organ. The gall-bladder on microscopic examination showed complete loss of surface epithelium, and in the innermost layers of the mucosa numerous large masses of micro-organisms were found, some of which resembled the typhoid bacillus morphologically and did not retain Gram's stain. It should be mentioned that the post-mortem examination was carried out 28 hours after death, and that organisms of the B. coli type were invariably associated with the B. typhosus. There was no evidence whatever that the typhoid carrier state had contributed to the fatal issue of the croupous pneumonia.

It is unfortunate that Kamm does not mention specifically whether the pneumonic areas in the lung were bacteriologically examined.

The last case to which I shall refer is that of Miss S., one of the female carriers reported by Watt (*vide* page 48). Operation was indicated in this case largely because of the depressed mental attitude of the patient, resulting from a knowledge of the numerous infections she had given rise to in others, and also because of the fact that for many years she had suffered from attacks of indigestion and biliousness. I am indebted to Mr. H. M. W. Gray, Surgeon, Royal Infirmary, Aberdeen, for kindly providing me with notes of the operation, which took place on November 12th, 1908.

The gall-bladder was only with great difficulty exposed owing to dense peritoneal adhesions in its neighbourhood (tuberculous peritonitis). It was of large size, and presented a diverticulum which occupied a recess in the liver. Two large gall-stones were found, from which the B. typhosus was recovered. Unfortunately the patient took the anæsthetic badly, and the contemplated removal of the gall-bladder and its diverticulum had to be given up.

Drainage was therefore resorted to as quickly as possible. Six weeks later the patient died from an aggravation of her lung trouble (phthisis pulmonum). Bacteriological examination of the bile invariably revealed typhoid bacilli. Unfortunately no examination of the fæces was made after the operation, so that it is impossible to say what effect drainage of the gall-bladder had on the excretion of typhoid bacilli. An autopsy could not be obtained.

I conclude this review of the operative treatment of carriers with the reflexion that no trustworthy evidence of permanent cure has so far been forthcoming, As I have indicated in a previous chapter, it may well happen that in cases of cholecystitis occurring at an early period in typhoid convalescence, surgical interference may be followed by a complete disappearance of typhoid bacilli from the fæces, but in long-established cases where the infective germs have acquired a permanent footing not only in the gall-bladder but also in the biliary tracts and duodenal recesses, the prospects of success by operation directed solely to the gall-bladder are extremely small. No definite verdict, however, on the efficiency or otherwise of this method can be pronounced until the cases so treated have been under bacteriological supervision for at least one or two years thereafter.

The treatment of intestinal carriers by typhoid vaccines has, it must be confessed, been unsuccessful or indecisive, though much was hoped from this method. As long ago as 1902 Koch suggested the employment of typhoid vaccines as a remedy for the carrier state. Thomson and Ledingham (loc. cit.) have treated five female carriers in this way with entirely negative results whether stock vaccines or homologous vaccines were employed. In most of the cases where serological examinations have been made, we have noted a marked rise in the agglutinins, but in a recent case treated solely with the homologous vaccine, no appreciable alteration took place in the agglutination-titre. In a recent report by the Director-General of the Army Medical Service (1909) an account is given of the vaccine treatment of an intestinal carrier Private L. both with stock and homologous vaccines, and a temporary cessation in the excretion of the germs in the stools was noted during the course lasting from February–May, 1909. Later, this case received X-ray treatment in the region of the gall-bladder. The carrier presented symptoms of slight cholecystitis, and this form of treatment might, it was hoped, bring about an increase of phagocytosis in the walls of an inflamed gall-bladder.

On 6th August, 1909, the treatment was begun, the gall-bladder region being exposed for three minutes to a 3-ampère current in a tube with a 6½-inch spark-gap. (Report by Cummins 1910.) Exposures were given three times a week for three weeks, and during this time and for three weeks afterwards the stools were typhoid-free. Positive results were, however, got on September 20th, 23rd, and continuously from October 4th to October 21st. Another X-ray course was commenced on October 20th and ended on November 15th. On November 1st the stools were positive, but examinations on November 5th, 9th, and 12th, and December 6th were negative.

The patient left hospital on November 16th. The outcome of this treatment, as perhaps showing some inhibitory action on the discharge of bacilli, is certainly interesting and suggestive, but at present it would be premature to signalize this method as affording a prospect of permanent cure. As a matter of fact the discharge of bacilli by the patient had been intermittent before commencement of the treatment. The method, however, deserves a further trial. The fæces and urine of this man, who is now a civilian, were examined by me on 3rd March, 1910, with negative results, but on June 4th, 1910, I. isolated the B. typhosus from his fæces, so that he cannot be considered as cured.

In another army carrier the method was tried but without the slightest effect on the discharge of typhoid bacilli.

In concluding this chapter we have to deplore the fact that so far the attempts to cure intestinal carriers have not yielded results affording convincing evidence of their success. It must indeed be admitted, on consideration of all the evidence adduced by the surgeon and the pathologist as to the nature of the lesions met with in carriers, that the problem of effecting a cure in these carriers is an extremely difficult one. In the case of carriers who are in an early stage of this condition, there may be some hope of effecting a permanent cure by one or other of the methods already tried and quoted above, but in long standing chronic cases the prospect of success of this kind would seem to be extremely remote.

It may be possible to diminish the number of carriers by some modification of the regime employed in the treatment of enteric cases. This is a question, however, on which I do not feel qualified to hazard any personal opinion, but it may be of interest to note that G. Mayer's (1910) observations led him to believe that an exclusive milk-diet during the disease and an abundance of milk during convalescence were most favourable in diminishing the crop of carriers. With a mixed diet on the other hand, the carrier-state was more likely to intervene. The data on which he founded this opinion (which is supported by Drigalski) were not, however, sufficiently extensive to admit of reliable conclusions being drawn.

I have already sufficiently alluded to the now universally recognised conception of enteric fever as essentially a septicaemic infection and to the animal experiments which show that the gall-bladder invasion occurs at an early stage through the medium of the blood and not by a later inroad from the intestine. Can we diminish or influence in any way the degree of this initial septicaemia? In the absence of an efficient antityphoid serum which would probably have this desired end, one can at present, in the light of certain experimental results obtained by Hilgermann (1909-*b*), only suggest the use of salicylates as a means of arresting or diminishing the typhoidal invasion of the gall-bladder and bile tracts.

The treatment of urinary carriers is discussed in the following chapter.

CHAPTER X.

Urinary Carriers.

Pathogenesis of the Condition and Treatment.

In the course of investigations on the carrier problem, urinary carriers have been met with much less frequently than intestinal carriers. The great majority of the chronic carriers discovered in connexion with endemic typhoid fever have been passing the typhoid bacillus in the fæces only and not in the urine. Certainly the typhoid bacillus is occasionally found in the urine of chronic intestinal carriers, especially of the female sex, but in these cases their presence in the urine is almost certainly due to fæcal contamination of the urine. When catheter specimens have been taken (in a few instances recorded) the urine has been invariably free from typhoid bacilli. I may here, however, give the data regarding the occurrence of B. typhosus in the urine of certain chronic intestinal carriers who have been examined over long periods.

1. From June, 1908, to July, 1909, the excreta of Mrs. H. (*see* Thomson and Ledingham (1910)) were periodically examined. The fæces were positive on 29 out of 34 occasions, while the urine was positive on 8 out of 26 occasions. On no occasion was the urine positive while the stools taken at the same time were negative. (*See also* Table at close of Chapter XII.)

2. "G." Female asylum carrier. Period of observation, September, 1907, to February, 1910. Fæces were examined on 41 occasions and were positive on 26. Urine was examined on 33 occasions and was positive on 4, viz., 24th September, 1908,

6th November, 5th December and 11th December. On one of these four occasions the fæces taken at the same time were negative, the explanation probably being that if the number of bacilli discharged is small and the urine happens to get contaminated, their demonstration in the latter is much less difficult. (*See also* Table at close of Chapter XII.)

3. "McC." Female asylum carrier. Period of observation, November, 1907, to February, 1910. Fæces were examined on 38 occasions and were positive on 31. Urine was examined on 29 occasions and was positive on 4 successive occasions, viz., 27th November, 5th December, 11th December, 1908, and 23rd January, 1909. (*See also* Table at close of Chapter XII.)

4. "C." Female asylum carrier. Period of observation, November, 1907, to February, 1910. Fæces were examined on 38 occasions and were positive on 13. (Intermittent carrier.) Urine was examined on 27 occasions and was positive on 1, viz., 5th December, 1908. (*See also* Table at close of Chapter XII.)

Carriers 2, 3 and 4 are isolated in the same building, and the coincidence of the dates on which the urines were positive would seem to suggest that about this period the samples of urine forwarded had become contaminated by fæcal material.

In chronic intestinal carriers of the male sex I have never found the urine positive.

Though in chronic intestinal carriers the occurrence of typhoid bacilli in the urine is rare, it is not so uncommon to find both fæces and urine infective in the acute or transitory carriers and in early convalescent carriers. In this connection reference may be made to the data regarding transitory carriers in Scheller's milk-spread epidemic. Some of these passed typhoid bacilli in the fæces only, some in the urine only, and some in both fæces and urine. In such cases one may suppose that there is a transient typhoidal septicaemia.

The true urinary carrier passes the typhoid bacillus in the urine only, and it is fortunate that such cases are so infrequently met with, as the danger of spreading infection by the urine is much greater than in the case of intestinal carriers.

The earliest cases of this condition were reported about the same time by Rousing (1898), Houston (1899), and Young (1900). It will be advisable to give an account of some of these cases in detail as they tend to throw some light on the pathogenesis of the condition.

Rousing's case was a male of 53 years who had had enteric fever 18 months previously. During the primary attack there had been no retention of urine and no necessity for catheterization. In convalescence the urine was milky and on standing, a heavy deposit formed. Several months later he began to have vesical pain and frequency of micturition especially at night. The urine was acid and contained pus but no blood.

The typhoid bacillus was present in large numbers in the urine. A suprapubic cystotomy was performed and the mucosa was found to have a dark red colour with numerous ulcerations separated by swollen rugae. Cloudy urine came from both ureters. The patient died one month after operation. At the autopsy the right kidney was enlarged and small abscesses were scattered about the kidney substance. The pelvis contained a stone of the size of a walnut and the pelvic mucosa was also swollen and ulcerated. The left kidney was in a similar but less marked condition, with millet seed abscesses in the cortex but no stone. There was no evidence of tubercle. Rousing believed that the calculus had been present for some time before the attack of typhoid fever, and that the trauma caused by it determined the localisation of the B. typhosus in the kidney.

It is known that some cases of nephrotyphoid may be associated with focal abscesses in the kidneys, but they are rare. (*Vide* Flexner (1896).)

Houston's case was a female of 35 years who presented no definite evidence of a primary typhoid infection. Three years before coming under observation she had been living in a house where two children died, one of bronchitis and the other of diarrhœa (patient's diagnosis). She had helped to nurse these children. Shortly afterwards she suffered from frequent micturition which, latterly, was accompanied by pain.

In 1895 (December) she had gone to hospital where she was treated for three months for painful and frequent micturition and occasional passage of blood. She returned to work, but in May, 1898, she again consulted a doctor who, after treating her for cystitis, sent her to hospital where her condition was thoroughly investigated. Her urine was turbid and opalescent, strongly acid and contained a small amount of albumen. The sediment contained numerous squamous cells with pus cells and bacteria. The typhoid bacillus was repeatedly recovered from the urine.

Her serum also agglutinated a known strain of the typhoid bacillus in a dilution of 1 in 100. She remained in hospital for six weeks without showing any abnormal temperature or other symptoms pointing to typhoid fever.

It was from this case that Houston drew the important conclusion that typhoid fever is a true general infection and not merely of local origin in the Peyer's patches of the intestines. This view was elaborated more fully by Horton-Smith (1900) in his Goulstonian lectures of the following year. This author held that the mesenteric glands are the primary seat of the typhoid infection, a view which by several years anticipated that of Forster. (*See also* Levy and Gaehtgens (1908).) With regard to the presence of the typhoid bacillus in the urine of typhoid patients Horton-Smith found positive results in 25 per cent. of cases examined. This is the percentage found in most recent work on the subject of typhoid bacilluria. Of 289 post-mortems in St. Bartholomew's Hospital on typhoid patients (during 30 years) Horton-Smith notes that abscesses of the kidney occurred only once. He believed that the typhoid bacillus grew in the bladder, the walls of which were chronically inflamed.

Young's case (also reported by Gwyn (1899)) was a male of 39 years who had had typhoid fever in 1893. On admission to hospital at that time he had shown pyuria, and one of his complaints was pain during micturition. Ten days after defervescence a relapse occurred but the pyuria continued. In 1897 the urine was very purulent and the B. typhosus was first isolated from it in 1898. His serum gave a positive Widal reaction.

In January, 1900, he returned to hospital when the urine was again positive. By cystoscopic examination a chronic ulcerative cystitis was found. There was also a secondary infection with the gonococcus, both gonococci and typhoid bacilli being isolated from the bladder. The fact that this patient also had syphilis in 1889 may have had some influence in predisposing the genito-urinary tract to particular and prolonged infection by the typhoid bacillus.

I have already referred to the cases of Irwin and Houston and Niepratschk, both of which were responsible for fresh cases of typhoid in their vicinity (Chapters III. and VI.).

Greaves (1907) reported the occurrence of a typhoidal pyonephrosis in a patient of 36 years who had had his primary attack of enteric six years previously. From the pain during micturition and the swelling in the left renal region a diagnosis of renal trouble was made and cystoscopic examination revealed that the left kidney was not functionating. At operation, a pyonephrosis was found with a phosphatic calculus in the left ureter. From the pus the typhoid bacillus was recovered in pure culture. Fourteen days after the operation the patient's blood serum agglutinated the typhoid bacillus in a dilution of 1 in 200.

Another important urinary case also associated with pyonephrosis and treated surgically with success was reported by Adrian (1908), and also more fully by Meyer and Ahreiner (1909). The patient was a girl who in 1897 (when six years of age) suffered from pyonephrosis. While under treatment in hospital for this condition she contracted typhoid fever, the pyonephrosis apparently antedating the typhoid attack. Shortly after her discharge home she infected a brother and both her parents with typhoid fever. For 10 years the girl remained perfectly well. Then gradually she began to suffer from acute pains in the left kidney region with swelling of left side of abdomen and thighs, and cloudy urine. A communication had evidently been established between the sac and the bladder, so that the typhoid bacilli vegetating in the sac were continually being discharged by the urine. In July, 1907, nephrectomy was performed. Typhoid bacilli were present in pure culture in the purulent contents of the sac. The actual seat of vegetation had very probably been an ulcerated area which was found near the ureteral orifice. Eight days after the operation bacilli were found in the urine, but afterwards it remained quite free of typhoid bacilli. Before operation the patient had given a Widal reaction of 1 in 10,000, but after operation the titre fell to 1 in 150.

These urinary cases bear a strong analogy to the gall-bladder cases, and therefore one would require to exercise the same caution in judging of the effect of operation. The absence of typhoid bacilli in the urine in the above case after operation certainly suggests that there were no secondary depôts in the bladder itself as one would have expected in such a chronic condition.

Sufficient data have not yet accumulated with regard to the organic lesions associated with the urinary carrier-state to enable us to formulate lines of treatment applicable to particular cases. It would be well, however, whenever opportunity offers, to examine these cases with peculiar care, employing the modern methods of cystoscopy and ureteroscopy, so that the sum total of definite knowledge on this subject may be increased.

That the principal depot of the B. typhosus may be a chronically inflamed or cystic renal pelvis is known, and this may or may not be associated with secondary depots in an inflamed bladder. The latter organ, however, is most probably the main depot in most cases, and the chronic typhoid bacilluria may be regarded as being due to some unwonted vulnerability of the bladder mucosa. The ordinary bacilluria occurring during the fever, may not be associated with any cystitis, but should retention occur, or should the bladder mucosa be more severely damaged than usual by the typhoid toxæmia, a more or less chronic unhealthy state of the mucosa is apt to be set up which may permit the indefinitely prolonged vegetation of the typhoid bacillus. Antecedent inflammatory conditions in the urinary tract may also, doubtless, predispose to the urinary carrier state.

We have not yet sufficient data to enable us to correlate the pathological findings met with in urinary carriers, with those found either in the so-called nephro-typhoid or in cases of acute typhoidal cystitis associated with definite lesions in the bladder. On this latter subject the articles by Schuder (1901) and Schaedel (1906) may be consulted. With regard to the kidney, Cagnetto and Zancan (1907) have shown that in most cases of typhoid fever, inflammatory foci (so-called lymphomata) with focal necroses or even minute abscesses are to be found. These, like the analogous small cell infiltrates described in the liver in fatal cases of typhoid fever, may also act as vegetation-depots of the typhoid bacillus, in the urinary carrier.

Treatment of Urinary Carriers.

Urotropin has proved so valuable in the bacillurias occurring during typhoid fever and in early convalescence from that disease, that one naturally anticipated a similar efficiency in urinary carriers. Such hopes have not been realised so far as the recorded reports show, and, perhaps, the reason of its failure is to be sought in the chronically inflamed and rugose state of the bladder, which may render the typhoid bacillus less readily accessible to its influence. Urotropin certainly has a marked temporary effect in reducing the number of bacilli excreted, but the latter return in full force when the administration of the drug is suspended.

The treatment adopted by Niepratschk (*vide* Chapter VI.) in his case, is so instructive that I give it in detail.

The carrier was isolated on 23rd February, 1908, and treatment with urotropin was at once begun.

First course of urotropin.

23rd February, 1908. Received 1 gm.

24th February–8th March. Received 2 gm. daily.

Result :—Soon after the commencement of the treatment the urine became clear and the number of bacilli decreased. On 26th February, the urine contained only 10,000 bacilli per cub. cent., and

and on 28th February none. On 17th March, when the remedy had been stopped for seven days, the bacilli were as numerous as ever.

Second course.

17th March–24th March. Received 3 gms. daily (=24 gms. in all), and a further 11 gms. up to 6th April.

Urotropin was then laid aside and hetralin—a compound containing 56 per cent. urotropin and 44 per cent. resorcin—was tried.

Hetralin course.

15th April–18th April	... ...	2 gms. daily.
19th April–16th May	... ...	3 gms. ,,
17th May–24th June	... ...	5 gms. ,,
25th June–2nd July	... ...	6 gms. ,,
3rd July–11th July	... ...	8 gms. ,,

The carrier's health was not affected by the drug. His appetite remained good. A reduction in the number of bacilli resulted, but it was not permanent.

Borovertin, a compound containing urotropin and boric acid (= hexamethylen tetramin triborate) was finally tried with apparent success.

Borovertin course.

23rd July–27th August. 6 gms. daily (= 1 gm. six times a day, and later 1·5 gm. four times a day).

This drug was not so well tolerated as the others. The patient suffered from loss of appetite and weakness, and his body-weight fell from 81 kilo to 70 kilo during the short period from 1st to 29th August. The drug was therefore stopped and the patient recovered very quickly. The disinfectant action on the urine was, however, remarkable. Two days after the first dose, bacilli were still present in considerable quantities, but, thereafter, none could be demonstrated by repeated examinations. Examinations on 27th July, 31st July, 3rd August, and many following days were negative. On 23rd October a litre of the urine was treated by the precipitation method employed in searching for B. typhosus in water, but the result was negative. As large a quantity as two litres was also examined without result.

On 1st November, 1908, Sergeant B. was considered "cured" and resumed his regimental duties. Weekly or bi-weekly examinations of his urine were still continued, but all were negative, and Niepratschk, writing in December, 1909, says that no further cases of typhoid fever have occurred in Sergeant B.'s regiment although he occupies his old position in the barracks.

What impresses one in the recital of this case is rather the systematic and persistent method which was adopted than the ultimate result. If this case may be chronicled as a "cure," it is a remarkable testimony to the efficacy of the drug treatment that a carrier of such long standing, presenting in all likelihood lesions of a chronic character in his urinary tract, should be so completely restored to a normal condition. Any further data regarding the after history of Sergeant B. will be welcomed. In the meantime it may be said that borovertin either alone or perhaps, preferably, after a preliminary course of urotropin, should be given a trial in urinary carriers.

Recently another cure by borovertin has been reported by Ustvedt (1910), but I have been unable to secure full details of the case. The carrier was a ship steward, who continually excreted typhoid bacilli in his urine. After a six weeks' course of borovertin (120 gms. altogether) the bacilli disappeared.

I now come to the treatment adopted by Irwin and Houston (*loc cit.*) in their female carrier case (*vide* p. 38).

For the first five weeks tonics and urinary antiseptics were tried, but with no effect on the bacilluria. Headaches and gastric pain were still complained of. Vaccine treatment was then resorted to, the homologous strain being employed in the preparation of the vaccine.

Course of Vaccine treatment :—

August 25th, 50 millions. Urine positive on September 1st.

September 2nd, 100 millions. Urine positive but bacilli less numerous on September 16th.

September 20th, 200 millions. Urine positive on September 22nd. Bacilli as numerous as ever.

The patient was now put on sodium lactate and the urine made alkaline.

Urine negative on October 8th.

October 9th, 300 millions. Urine negative on October 20th.

October 23rd, 500 millions. Urine negative on October 24th. Clear with no pus and no albumen. Urine and fæces negative on November 21st.

November 21st, 1,000 millions. Urine negative on November 23rd. Fæces negative.

During the treatment, the headaches, gastric pain and feeling of tiredness disappeared, and the patient gained markedly in weight. (The last examination which was negative was made on April 24th, 1909 (Houston (1909)).

The authors believed that by rendering the urine alkaline the typhoid bacilli became more susceptible to the action of the immune bodies developed in the serum by the vaccinations.

Houston (1909) has treated two other urinary cases with typhoid vaccine, but a definite report cannot yet be given.

Two chronic urinary carriers invalided from the Indian army have been treated with typhoid vaccines (Fawcus, Kennedy and Cummins (1910)) in this country, but no definite improvement in their condition was realised. These cases had previously been treated with urotropin without effect, but it was noted that when a diuretic ("sanmetto") was given in combination with the urotropin, a marked fall in the bacilluria took place and the purulent deposit became almost nil. On cessation of the treatment, however, the bacilli were discharged again in large numbers. Major Cummins suggests that more definite results might be attained by combining sanmetto and urotropin with vaccine treatment; but in the light of Niepratschk's results it may be contended that it is the persistent pushing of treatment over a prolonged period that is most likely to be of value in the end.

CHAPTER XI.

DIAGNOSTIC METHODS EMPLOYED IN THE SEARCH FOR CARRIERS.

The demonstration of the typhoid bacillus either in the fæces or in the urine, is essential before a person can definitely be certified to be a carrier.

In dealing with large institutions, however, where enteric fever has been endemic, some indication may be got of the presence of carriers by carrying out an extensive series of Widal tests, commencing with those subjects who are ascertained to have suffered from typhoid fever in previous years or who may happen to present symptoms of gall stone trouble.

It is stated by Kayser (1909) that about 75 per cent. of carriers give a well-marked Widal reaction, but I am inclined to think that this percentage is rather high, if agglutination at 1 in 100 be taken as the criterion of a positive Widal reaction. Of nine chronic carriers whom I have personally observed, five gave a definitely positive reaction, while four gave an incomplete or absolutely negative reaction. On the other hand, in institutions, a positive reaction is occasionally obtained in persons whose fæces, though repeatedly examined, fail to show the presence of the typhoid bacillus. Certainly in such cases, we have to reckon with intermittent periods, imperfect bacteriological methods and possibly other factors not yet fully understood. Kamm (1909) had an experience of the Widal test which was unfortunate and perhaps exceptional. He commenced the search for carriers in an asylum by the examination of 136 blood samples. Four of these gave a reaction of 1 in 50, one gave a reaction of 1 in 100, and three gave a reaction of 1 in 200. Of these eight persons, four had had enteric fever about eight or twelve years previously, while in the other four, there was no record of typhoid. Castor oil was given to these eight persons, and their fæces repeatedly examined thereafter, but in none of them could he demonstrate the typhoid bacillus. It would be interesting to know the further history of these cases and whether definite carriers were ultimately discovered.

Eccard (1910) in the course of his examination of an asylum (*vide* Chapter V.) commenced by performing Widal tests in all departments of the building. The figures obtained he does not record, but he was able to classify the inmates into three groups :—

1. Those who gave no Widal reaction.
2. Those with Widal reaction of 1 in 50.
3. A small number who almost always gave a reaction in 1 in 100. The stools of this group were frequently examined and in five of them he was able to demonstrate the typhoid bacillus. In three others of this group who frequently gave a reaction in 1 in 100, he could never demonstrate typhoid bacilli in the fæces.

An important point noted by him in the course of this investigation was that in a large number of those who were Widal-free or who occasionally gave a reaction in 1 in 50, the titre would suddenly spring to 1 in 100 with symptoms of intestinal disturbance, vomiting and diarrhœa. After the attack the titre again fell.

Some trouble was experienced with three severe tubercular cases who gave positive Widal reactions. One of these died but there were no signs of typhoid fever. (He makes no mention of the condition of the gall bladder.) This question of positive Widal reaction in cases of tubercle is discussed in Chapter XII.

Eccard, however, thinks that, at least as a preliminary step, the Widal test should be employed in large institutions, a titre of 1 in 50 to be regarded with caution and one of 1 in 100 as suspicious. He does not appear to have met with carriers who give practically no reaction even in 1 in 20.

When proper bacteriological conveniences are available, the best method of procedure is the immediate examination of the excreta of all persons concerned, commencing with those who give some history of having passed through a typhoid attack or who suffer from gall stones, and then passing on to the others. At the same time Widal tests should be carried out and repeated bacteriological examinations made at intervals, in those cases which give a positive Widal reaction.

It is impossible here to describe in detail the various media employed or recommended for the isolation of the typhoid bacillus from the excreta. For those interested in this subject a list of these media will be found at the end of this chapter.

Amidst a plethora of methods and modifications of methods, all of which are probably excellent in the hands of those accustomed to them, it seems to me of far more importance to obtain a thorough knowledge of the potentialities and the working efficiency of one medium, than to be continually changing from one medium to another without a clear knowledge of the advantages to be derived by the change. Many of the newer media introduced for the isolation of the typhoid bacillus have been recommended strongly on their merits as inhibitors of the B. coli group, but the data on which such judgments are based, have usually been obtained by practice with artificial mixtures of B. typhosus and B. coli, to which the particular medium or inhibiting agent in the medium is added, and not with the actual fæces of typhoid patients or carriers. The writer's experience has been confined to the bile-salt-lactose-agar medium of MacConkey (1901), with neutral red as indicator. This medium has been in constant use for many years and has proved eminently satisfactory. The actual method is briefly as follows:

For Fæces.—About two to three large loopfuls of the fæces are emulsified in broth and allowed to sediment for an hour. From the upper portion of the fluid, two small loopfuls are removed and spread by means of a bent glass rod over two or three Petri plates containing the solidified medium, which should be quite dry. On the following day, colourless colonies suggesting the typhoid bacillus are picked off and inoculated into mannite-peptone-water. If acid only is formed in any tube or tubes and no gas, the probability is strong that the organism is the typhoid bacillus (or the dysentery bacillus—Flexner-type), but it must be remembered that the fæces contain a small group of organisms whose characters in some particulars, present a strong similarity to those of the B. typhosus (Morgan and Ledingham (1909)). As a rule these organisms give little trouble and can be readily differentiated by the application of fermentation and agglutination tests.

The B. typhosus gives an acid reaction without gas formation on glucose, mannite and sorbite ; a late acid reaction on dulcite (Penfold (1910)), (Grattan (1910)) and no reaction on cane sugar or lactose. Indol is tested for by Ehrlich's method (*vide* Marshall (1907)) after four days' growth on broth, and finally an agglutination test is made with a potent antityphoid serum which agglutinates a known strain up to 10,000 or 20,000.

Some workers commence the investigation of a suspicious colony by performing an agglutination test, but it frequently happens that typhoid bacilli taken directly off the plate and tested with an antiserum, give a very feeble and unconvincing reaction. Scheller (1908) has observed quite a gradation of agglutinability in typhoid colonies taken directly from the same plate. Media containing certain inhibiting agents like malachite green, have been found to reduce the agglutinability of typhoid bacilli when examined directly.

By performing the agglutination test after sub-culture on broth. all the strains which I have isolated from MacConkey plates (either from fæces or urine) have without exception agglutinated up to 1 in 5,000 or 1 in 10,000.

Further, by preliminary performance of the fermentation tests, one acquires a knowledge of the characters of the many members of the non-lactose group whose appearance on plates may simulate strongly that of the typhoid colony, which may indeed give a slight reaction with a potent antityphoid serum, but which can be absolutely excluded by their fermentation and other properties.

A motile non-lactose-fermenting organism producing a permanent acidity on litmus milk and no indol, rendering glucose, mannite, and sorbite, but not cane sugar, acid, has invariably satisfied the agglutination test when finally applied. The rare occurrence of a non-motile or temporarily non-motile typhoid strain has been reported on at least two or three occasions. Those recorded recently by Ernst (1908) and Fischer (1909) ultimately recovered their motility after cultivation on various media. Such a strain I have also encountered on one occasion. It gave all the fermentation tests and the agglutination and absorption tests, but in spite of various methods of culture and animal passage, it has not so far given evidence of motility. The strain referred to was obtained from the stools of a female lunatic in the course of investigations in an asylum. Further samples of fæces were negative, and it seems quite possible that this woman had been a so-called temporary or acute carrier.

It has already been mentioned that frequently the stools of typhoid carriers give practically pure growths of B. typhosus on plates, and there is little trouble, therefore, in isolating them in such circumstances. I have not observed that on these occasions the stools are unusually loose in character, but on the other hand there seems to be no doubt that after a period of constipation it may be possible to encourage the appearance of the bacillus in the fæces by the administration of a mild purgative or cholagogue.

The isolation of B. typhosus from the urine in urinary carriers, is, as a rule, very easy, as it is generally the only organism present. Some difficulty is said to have been encountered in getting organisms isolated from urine to agglutinate properly, but this has not been my experience.

The diagnosis of carrier cases by serological methods (opsonins or tropins, or complement-deviating bodies) can only be regarded as adjuvants to the ordinary bacteriological routine. This subject will be discussed in Chapter XIV. (*see also* Hamilton, 1910).

Finally, I may refer to an interesting, though not very practical, method of demonstrating the presence of B. typhosus in a carrier who may not at the time be actively shedding the bacillus in the fæces. Weber (1908) passed a dose of oil (about 200 c.c.) into the stomach, thereby causing a regurgitation of typhoid-containing bile into that organ. After half-an-hour the stomach was emptied and a fluid obtained which presented an upper oily layer and a lower watery bile-stained layer. In two chronic carriers whose stools contained only a very few typhoid bacilli, he was able to recover the typhoid bacillus from the upper oily layer. It is not always possible, however, to obtain this back flow of bile into the stomach.

Media employed for the Isolation of B. Typhosus from the Excreta.

It would take too much space to discuss the respective merits of the many media employed for this purpose, but some reference will be made to the results recorded by those who have had personal experience of their use, or who have instituted comparative tests as to their efficiency. For the preparation of the various media the reader is referred to the original papers (*see* Bibliography).

Only those media which are in current use at the present time will be referred to.

1. Bile-salt-lactose-neutral-red-agar.

MacConkey's (1901) original media did not contain the neutral-red. The latter was added on the suggestion of Grünbaum and Hume (1902).

For full information regarding the development of bile-salt media, the reader is referred to a recent communication by MacConkey (1908), in which the exact mode of preparation is described.

2. Medium of Drigalski and Conradi (1902). Lactose-litmus-nutrose-kristalviolett-agar.

The preparation of this well known medium is described in most bacteriological text-books. On the Continent its use is now very largely superseded by media of more recent development. (*See* below).

3. Medium of Endo (1903). Lactose-fuchsin-sodium-sulphite-agar.

4 Method of preliminary enrichment by caffeine. Roth (1903), Ficker and Hoffmann (1904), Lubenau (1907), Werbitzki (1909).

The fæcal material is incubated in the caffeine enriching solution and finally plated on Drigalski-Conradi plates.

Caffeine has also been added with advantage to fuchsin media (Gaehtgens, 1905), and malachite-green media (*see* below).

5. Media containing malachite-green, introduced by Loeffler (1903) and since modified and improved by himself and others.
   - (*a.*) Malachite-green-nutrose-agar. Loeffler (1906).
   - (*b.*) Malachite-green-bile-agar. Loeffler (1907).
   - (*c.*) Malachite-green sodium sulphite-bile-agar. Padlewski (1908).
   - (*d.*) Malachite - green - safranin - reinblau media. Loeffler, Walter, Dibbelt, and Wehrlin (1909).
   - (*e.*) Method of Lentz and Tietz (1903, 1905) in which the malachite-green medium is used mainly as an enriching medium, the growth being subsequently plated on Drigalski-Conradi or Endo plates.

(This is the method in current use at the Strassburg Institute) (*see* below).

6. Brilliant-green-picric-acid-agar. Medium of Conradi (1908).

Fawcus (1909) has modified this medium by adding to it bile-salt and lactose.

7. China-green-agar. Medium of Werbitzki (1909).

During the last two years some important comparative trials have been made of these various media, but, as might have been expected, the results have shown little unanimity. It would appear, indeed, that the media which prove most efficient in these trials are those with which the various authors themselves have had most practical experience. The results certainly bear out the statement made earlier in this chapter that a thorough knowledge of the capabilities of these media under all conditions is essential before one can arrange them in an "efficiency-scale." It may be of interest, however, to cite some of these results as they may prove of value to workers in this field.

Schumacher (1909*b*) has compared the efficiency of the recently-introduced Conradi medium (brilliant-green-picric acid) with that of Endo, employed either alone, or after enrichment on malachite-green.

The latter was the method then in constant use at the Institute in Trier.

500 samples of excreta (suspected to contain B. typhosus) were plated on these two media simultaneously, the amount of material plated being the same in each case.

Results :—

Endo plate alone ...	45 positive results	= 9 per cent.
Endo plate + malachite-green enrichment ...	50 ,, ,,	= 10 ,,
Brilliant - green picric acid (Conradi) ...	54 ,, ,,	= 10·8 ,,

So far as the percentages go, the differences do not appear very great, but the author on reviewing his results came to the conclusion that the brilliant-green plate materially increased the chances of obtaining positive results. However, in view of the fact that brilliant-green sometimes failed where Endo or malachite-green succeeded, he advised the following combination of the two methods. The material is spread on a brilliant-green plate, then with the same bent rod on a malachite plate, and from this again on an Endo plate.

The majority of the positive results would be got on the Endo or brilliant-green plate, and this may be still further raised by washing off the growth on the malachite plate and replating. This three-plate system is at present in use at Trier, and experiments are being made with the object of combining malachite-green and brilliant-green in one plate.

Kathe and Blasius (1909), of the Hygiene Institute in Halle, have reported on a series of comparative tests made with the following media :—

Malachite-green-Drigalski (Lentz-Tietz).
Endo.
Brilliant-green-picric acid (Conradi).
Malachite-green-bile-agar (Padlewski).

The material employed was the fæces and urine of patients, in whom a definite diagnosis of enteric fever had been made, either by successful isolation of bacilli from the blood, or by the Widal reaction. Samples were taken at various stages of the disease, and both freshly passed material and material several days old was employed. Of each medium, one large or three small plates were spread, with approximately the same quantity of material. The plates were examined after 18–22 hours at 37° C. and again after a further period of 20–24 hours. Attention was paid only to the presence or absence of typhoid colonies and not to their relative numbers. 53 samples in all were examined, viz., 22 of urine and 31 of fæces. With the latter they obtained the following results :—

Malachite-green-Drigalski (Lentz-Tietz)	3	positive	results	= 10	per cent.
Drigalski-Conradi	6	„	„	= 19	„
Endo	8	„	„	= 26	„
Brilliant-green-picric (Conradi)	9	„	„	= 29	„
Malachite-bile (Padlewski) ...	10	„	„	= 32	„

On the whole, therefore, the media of Padlewski, Conradi and Endo proved most satisfactory, whereas the method of Lentz-Tietz proved least successful in this particular trial although the authors had carried out exactly the directions recommended by its originators.

As so many others have found, Kathe and Blasius were unable to satisfy themselves of the favouring action of malachite green on the typhoid bacillus and its inhibitory action on B. coli. In their view, the main cause of the discrepancy in the results with malachite green, is to be found in the varying resistance offered by typhoid strains to this agent, some being able to grow on it readily while others are completely inhibited.

There is no one "medium of selection" for the isolation of B. typhosus from fæces. Each medium has its advantages and disadvantages and in order to obtain the maximum efficiency Kathe and Blasius recommend, as Schumacher does, a combination of three media, viz., Padlewski, Endo and Conradi (brilliant green picric acid) with subsequent plating on Endo.

The method they adopt is the following:—Two or three loops are spread on a Conradi plate (brilliant green) and with the same rod, a Padlewski plate is spread.

Two to three loops are also spread on a malachite-green plate and with the same rod an Endo plate is spread. The plates are incubated for 18–20 hours, and if no suspicious colonies are present the Conradi and the malachite-green plates are washed off and plated again on Endo. They are then left for another period of 18–20 hours.

(For information regarding the cost of these media, this paper by Kathe and Blasius should be consulted.)

Other observers, Grimm (1909), Fischer (1909), &c., have reported very favourably on the method of Lentz-Tietz. Thus the former obtained the following results with 25 samples of fæces from typhoid patients or carriers:—

Drigalski-Conradi	9	positive cases	= 36 per cent.
Malachite-bile-agar (Loeffler)	9	„ „	= 36 „
Padlewski	12	„ „	= 48 „
Brilliant-green (Conradi) ...	14	„ „	= 56 „
Lentz-Tietz	15	„ „	= 60 „

Fischer's material was 100 samples of fæces which certainly contained typhoid bacilli.

With the methods of Lentz-Tietz, Endo and Conradi (brilliant green) he obtained positive results in 76 per cent., 63 per cent. and 48 per cent. respectively.

Megele (1909), on the other hand, working with the fæces of carriers found Padlewski's method the most efficient. His figures were:—

Drigalski-Conradi ...	70	per cent. positive results.
Conradi (Brilliant-green)	66·6	„ „ „
Malachite-green (Loeffler)	33·3	„ „ „
Padlewski	79	„ „ „

The most recent comparative tests have been made by Gaehtgens and Brückner (1910) of the Strassburg Institute, where the method of Lentz-Tietz is in constant use for the isolation of typhoid bacilli from excreta.

One hundred samples of fæces were examined, viz., 22 from carriers, 72 from typhoid patients, and six from paratyphoid patients during the febrile period. They were plated simultaneously on seven different media with the following results:—

Lentz-Tietz	66	positive	= 66 per cent.
Conradi (Brilliant green) with subsequent washing off	59	„	= 59 „
Caffein-Fuchsin-Agar (Gaehtgens)...	58	„	= 58 „
China-green-Agar (Werbitski) ...	53	„	= 53 „
Fuchsin-Agar (Endo)	50	„	= 50 „
Malachite-green-sodium sulphite-bile-agar (Padlewski)	48	„	= 48 „
Conradi (brilliant green) without subsequent washing off	41	„	= 41 „

The non-success, which Kathe and Blasius had with the method of Lentz-Tietz, they attributed to the fact that the plates had not been incubated long enough. According to Gaehtgens and Brückner the "enrichment" plates should be incubated for at least 24 hours and if only slight growth occurs, for another period of 24 hours. Kathe and Blasius had also used a neutral agar, whereas Gaehtgens and Brückner employed one with a reaction of 1 per cent. normal soda below the Phenolphthalein neutral point. (Modification by Klinger, 1904.) It is certainly significant that the medium with which Gaehtgens and Brückner had had constant experience for five years in the Strassburg Institute should have stood this comparative test so well.

CHAPTER XII.

Discussion of the General Question of Carrier-Infectivity.

This question involves the consideration of many factors which must play a part in the successful infection of a new host by a carrier. In the first place, the bacillus has to be transferred directly or indirectly from the carrier to the new host. The degree of likelihood of this transference taking place will depend, perhaps largely, on the personal hygiene of the carrier and the measures of disinfection adopted by him, provided he is cognisant of his condition, and on the method of disposal of his stools and urine. Where the carrier is unaware of his condition, the chances of successful transference are much greater in spite of average personal hygiene, and they are also very much intensified in the case of lunatics of unclean habits. Further, much will depend on the environment and personal hygiene of the new host in so far as these conditions affect his liability to exposure to infection. If these conditions are satisfied, *i.e.*, given carriers and associates of uncleanly personal habits, the probability of successful transference to a new host is greatly enhanced, provided the carrier is actively excreting the typhoid bacillus. Throughout this memoir numerous instances of prolonged intermission (up to two years in some cases) are referred to. Certainly, some of these may be only apparent intermissions, *i.e.*, the bacilli discharged may be so few that our bacteriological methods cannot reveal them. There can be no question that many of the instances of intermittent infectivity cited throughout the earlier chapter of this memoir, are to be explained by the variation in the numbers of bacilli excreted by the carrier from time to time. Obviously the more numerous the bacilli excreted are, the greater is the chance of their being successfully transferred to a new host.

The magnitude of the dose received by the new host is probably a most important factor in determining whether a subsequent infection takes place or not.

Given a successful transference to the new host, there remain for successful infection at least two further factors, viz., virulence of the strain and the individual susceptibility of the new host. With regard to the first, a general notion has prevailed that carrier strains are less virulent than those from acute typhoid cases. As to this there is no sufficient evidence. If, as some authors have done, we take the mortality among the affected as the criterion of virulence, we find entirely discordant conclusions. If, on the other hand, the effect of the bacillus typhosus on animals be selected as an index of its virulence, the evidence hitherto available on this point is found to be inconclusive. As a matter of fact, seeing that the experimental reproduction of enteric fever in animals is impossible, we possess really no reasonably certain experimental criterion of virulence. Intraperitoneal inoculation of the guinea-pig has, however, been widely employed in testing the virulence of typhoid strains, and the variations in the magnitude of the minimal lethal dose for that animal have been shown to bear some relation to the ease with which the bacillus is acted upon by the various antibodies in normal and immune serum (lysins and opsonins).

So far as carrier strains have hitherto been compared with those isolated from typhoid cases in respect to their virulence for the guinea-pig, no marked divergence has been found. The same variations in virulence are met with among carrier strains as among those from acute cases.

Lentz (1905) sent 20 carrier strains to the Institut für Infektionskrankheiten to be tested for virulence, with the result that neither a greater nor a less degree of virulence than that of ordinary typhoid strains could be detected. It cannot be said, however, that enough strains have yet been examined in this respect to justify definite decision on the matter. Most authors have omitted to test the strains they have isolated on the guinea-pig. It is highly important that in performing such tests, as small an interval as possible should elapse between isolation of the strain and the determination of its virulence. I have adopted the following technique in testing the virulence of carrier strains for the guinea-pig.

The fæces are plated in the usual way, and on the following day, if typhoid-like colonies are present, one is touched with the point of a straight needle and inoculated into a fixed quantity of broth (6 c.c.), which is then incubated for 48 hours. A control mannite tube from the same colony is also inoculated, so as to make certain that the colony is really a typhoid colony. Growth for 48 hours in this way gives a fairly constant number of bacteria per cubic centimetre (700 to 800 millions). Guinea-pigs of 250 gram. weight are then inoculated intraperitoneally with falling quantities of the broth culture. The result has been that most of the strains tested kill in 1 c.c., a few in 0·1 c.c., and a few in 2 c.c. There is therefore no evidence of lack of virulence.

Far more important than the isolated virulence test is the periodical test, as there is certainly the possibility that the virulence of the strain may vary at different times of the year in the same carrier. My observations on this point are not on a sufficiently large scale to permit of trustworthy conclusions being drawn, but some results already obtained suggest that some such variation in virulence occurs. For example, a strain isolated from an intestinal carrier on 23rd October, 1909, killed in one-tenth c.c. A culture isolated on 19th November killed only in 1 c.c. On 1st February, 1910, the culture isolated did not cause death in 1 c.c. On 14th February it did not kill in 2 c.c.

However, without much further investigation it would be unjustifiable to assume that the virulence of a carrier strain is much reduced during the winter months. Whether the guinea-pig's susceptibility to the B. typhosus varies in the opposite direction according to the season must be left undecided.

That an avirulent bacillus might suddenly regain its virulence was suggested also by Scheller (1909), who, however, gave no evidence in support of this point.

Niepratsck (1909) who made a very careful examination of the strain isolated from the urine of a urinary carrier (*see* Chapter VI.), found that its virulence for the guinea-pig was distinctly low. By passage through the guinea-pig he succeeded in raising the virulence considerably, and he suggested that a similar increase of virulence might be attained by passage through the human body, and in this way, slight or unrecognised infections might be followed by infections of average severity.

I now come to the second factor involved in the successful infection of a new host by a carrier, viz., the susceptibility of the host. Unfortunately we have no satisfactory method of experimentally estimating the strength of this factor, and our conception of its importance must depend for some time on actual observed facts. Thus we have seen that the typhoid bacillus may be taken into the intestine, may even gain entrance to the general circulation and be passed in the urine without giving rise to any obvious infection at the time. Scheller's (1908) cases in the milk-spread epidemic already quoted (*vide* Chapter IV.), afford one of the most convincing instances of this phenomenon. In view, however, of the demonstration of typhoid bacilli in the fæces and blood during the incubation stage of typhoid fever by Mayer, Conradi and others, it might be expected that some of these so-called temporary carriers would ultimately present symptoms of infection, and Mayer (1910) has actually observed such cases.

It seems appropriate here to refer to certain observations of Busse (1908) on the occurrence of typhoid bacilli in the blood of patients at whose autopsy tubercular lesions only were found.

The first case was a female of 48 years who was suspected to be suffering from typhoid fever. She was admitted to hospital on 31st January, 1907. The typhoid bacillus was twice isolated from the blood, viz., on 1st February and 6th February, 1907, but the serum gave a negative Widal reaction. Death took place on 7th February, 1907. Post-mortem, most of the organs showed miliary tubercules, the lymph glands were caseous, but no lesions pointing to a typhoid infection were detected (Peyer's patches not swollen). The organs, however, were not examined bacteriologically, and no mention is made of the condition of the gall-bladder. Further, what appears to me a point of importance, was the fact that rose-spots were present on the fourth day of her sojourn in hospital. There was no evidence of her having had a previous attack of enteric fever. The second case was also a female of 25 years suspected to be suffering from typhoid fever, who was admitted to hospital on 9th April, 1907. The Widal reaction was negative, but the typhoid bacillus was isolated from the blood on 10th April, 1907. The spleen was palpable under the ribs and apparently also tender. Death took place on the 16th April, 1907. At the autopsy generalised tuberculous lesions were present in the lungs, intestine, peritoneum, &c. There was no swelling of the Peyer's patches, but three ulcers with tuberculous areas at their bases were present in the small intestine. The spleen, lungs, liver, bile and mesenteric glands were bacteriologically examined with negative results. A typhoid-like bacillus was, however, isolated from the bile, but it was not thoroughly examined, which is unfortunate.

In Busse's view, the typhoid bacillus was living a saprophytic existence in these two cases, as in those carriers who have never had any clinical symptoms of a typhoid infection. The blood invasion, he believed, was brought about by an increased permeability of the intestinal wall owing to the tuberculous lesions therein. A phthisical female patient in whom there was no suspicion of typhoid fever was also examined. From her blood the typhoid bacillus was isolated on several occasions although the Widal reaction proved negative. The urine and fæces of this case were examined bacteriologically with negative results.

Further, from the blood of a case of pneumonia the typhoid bacillus was isolated although the urine and fæces were negative. This patient presented symptoms of diarrhœa at first, but the course of the illness proceeded as a typical pneumonia of the right lower lobe. No evidence is afforded, however, proving that the lung infection was not of a typhoidal nature.

Busse's conclusion was that all these cases were probably carriers although they presented no history of having had typhoid fever. The typhoid bacillus, leading a saprophytic existence in the intestine, reached the blood stream as a result of an increased permeability of the intestinal muscosa due to the tubercular lesions. Further, he concluded that the presence of typhoid bacilli in the blood is not an absolute sign of the presence of a typhoid infection.

These results of Busse have to be considered in the light of accumulated knowledge regarding atypical and apparently symptomless enteric fever on the one hand and typhoid carriers with no typhoid history on the other hand. The explanation given by Busse that these cases were really typhoid-carriers, and that a passive invasion of the blood-stream took place as the result of the intestinal lesions, may be a perfectly sound one, but there is nothing in the evidence which entirely excludes the possibility of these cases being really mixed infections of tubercle and typhoid.

In chronic carriers the blood has very seldom been examined bacteriologically, but in the few examinations made, it has invariably been negative. During an exacerbation of cholecystitis, however, the blood may be found to contain the bacilli.

Some years before Busse's observations on this question appeared, Jurgens (1907) had reported the finding of typhoid bacilli in the fæces of a tuberculous patient in the course of an epidemic of typhoid fever. A diagnosis of enteric fever was made, but from the further course of the disease there was no doubt that the symptoms were attributable entirely to the tubercular infection. V. Krehl (1906) also recorded the discovery of typhoid bacilli in the blood of a case which proved at autopsy to be one of miliary tuberculosis. All such cases demand a more thorough bacteriological investigation than has hitherto been accorded them, and it would be advisable in cases of suspected miliary tuberculosis in children to examine whether the blood serum gives a Widal reaction and whether the typhoid bacilli can be isolated from the blood. The literature contains not a few references to the presence of agglutinins for the typhoid bacillus in the serum of tuberculous patients (Jurgens (1907), Krencker (1909) and others) but the most recent observations of Roth (1910) on this subject go to show that such occurrences must be very rare. The latter observer examined the sera of 100 cases of severe pulmonary tuberculosis, including five cases of the miliary form. The dilutions he employed were 1 in 50, 1 in 75, 1 in 100, and 1 in 200. Of this large series, five only gave a reaction in 1 in 50 and one in 1 in 200. This latter case was a man of 44 years who had never suffered from typhoid fever. Five years previously he had had an illness diagnosed as "Influenza." What most of his predecessors in this field had neglected to do, Roth did; he made repeated examinations of this person's fæces, but so far he has been unable to isolate typhoid bacilli therefrom.

Several authors have reported instances which suggest that certain factors such as trauma, pregnancy, the puerperal state, &c. may predispose to infection. Thus Levy and Wieber (1907) report the case of a woman who had her first child on 1st October, 1906. and 12 days later had recovered from the effects of confinement. On 21st October she fell ill with typhoid fever, and the bacillus was found in her fæces. The mode of infection was doubtful, but it was found that her mother had stayed with her in the house since the birth. The mother, who was quite healthy, belonged to a village in which enteric cases had been notified in 1906. During the summer she had had an influenzal attack, which was now judged to be typhoid fever. Her stools were therefore examined and she was found to be a carrier. Thus the woman was apparently infected by her mother, and in Levy and Wieber's view, childbed was the predisposing cause.

Cases of enteric fever in the puerperium have not infrequently been diagnosed as puerperal fever. Levy and Wieber report in illustration of this point, that two epidemics occurred in two villages near Strassburg, the one with 14 cases and the other with 11 cases. They were both ascribed to contact with a typhoid-sick woman in the puerperium whose illness had not been diagnosed as such, but as puerperal fever. Recently I had an opportunity of examining the blood of the umbilical cord, at the confinement of a typhoid carrier who had been under observation for over a year. The typhoid bacillus was recovered from the blood, but organisms of the Coli group were also present so that some faecal contamination of the blood, while the sample was being taken, could not definitely be excluded. The examination was undertaken in order to determine whether the general disturbance incidental to pregnancy and childbed might induce a general typhæmia. Unfortunately, for the reasons above mentioned, the matter remains not proven. As it was, the child remained quite healthy and the mother made a normal recovery.

Another factor has been insisted on by Conradi, viz., that strangers are most ready to contract infection when they come for the first time in contact with carriers. This proposition is a fascinating one and certain facts observed in connection with outbreaks due to carriers may be regarded as lending it some support.

For example, we have noted that the victims of the New York cook (reported by Soper, *vide* Chapter III.) were frequently new servants who had only quite recently come in contact with her, while the members of the household, who had been served by her for a considerable time, escaped infection. It would be quite legitimate to suppose that the latter had gradually acquired a certain degree of immunity by the ingestion of repeated small doses of typhoid bacilli, while the new servants not so protected would more readily react to infection. There is, however, the further possibility that, owing to the closer contact of the servants with the carrier, the less strict cleanliness in force in the servants' hall at mealtimes and otherwise, and the use of the same privy, the dose of bacilli ingested by the servants would be liable to be greater during any given period than that ingested by the other members of the household.

Davies and Walker Hall (1908) have put forward the view that it is only at certain seasons that carriers are effective and that for every carrier there is a particular season when infection from him or her is most likely to occur. The evidence on which this view is based is not convincing. That carriers generally are more likely to give rise to infection during the summer and autumn months has been frequently commented on and is exactly what might be expected, in view of the greater chances that the typhoid bacillus has, during this period, of prolonging its extra-corporeal existence and multiplying itself before gaining entrance to a fresh host. Intermittency in the excretion of the bacillus by a carrier is certainly not always confined to the same season of the year and may indeed not be observed at all even when the carrier's excreta are frequently examined over a long period. Thus in the Brentry case, Davies and Walker Hall found that the fæces of Mrs. H. were negative during January, February and March of 1908 (three examinations). Further examinations of the fæces and urine of this carrier were carried out by me during the period June, 1908–July, 1909 (Thomson and Ledingham (1910)), and the results are recorded in the annexed table with similar data regarding other carriers who have been under bacteriological supervision for considerable periods.

TABLE showing results of examinations of fæces of chronic carriers (Urine results not shown).

—	Mrs. H.		"G."		"McC."		"C."		"W."		"Ca."		"P."	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
1907.														
Sept. ...	—	—	1	—	—	—	—	—	—	—	—	—	—	—
Oct. ...	—	—	1	1	—	—	—	—	—	—	—	—	—	—
Nov. ...	—	2	1	—	2	—	1	—	—	—	—	—	—	—
Dec. ...	1	—	?	—	?	—	?	—	—	—	—	—	—	—
1908.														
Jan. ...	—	1	1	—	1	—	—	1	—	—	—	—	—	—
Feb. ...	—	2	?	—	?	—	?	—	—	—	—	—	—	—
Mar. ...	—	—	—	1	1	—	1	—	—	—	—	—	—	—
April ...	1	—	?	—	?	—	?	—	—	—	—	—	—	—
May ...	?	—	?	—	?	—	?	—	—	—	—	—	—	—
June ...	5	—	—	1	1	—	1	—	—	—	—	—	—	—
July ...	4	—	?	—	?	—	?	—	—	—	—	—	—	—
Aug. ...	2	—	1	—	1	1	—	3	—	—	—	—	—	—
Sept. ...	4	—	1	3	—	1	—	1	—	—	—	—	—	—
Oct. ...	3	—	2	3	3	2	—	5	1	—	1	—	—	—
Nov. ...	2	1	2	1	3	—	1	2	?	—	?	—	3	1
Dec. ...	1	1	2	—	2	—	1	1	1	—	1	—	?	—
1909.														
Jan. ...	1	1	1	—	1	—	1	—	1	—	—	1	?	—
Feb. ...	2	1	2	1	1	2	1	2	?	—	?	—	2	—
Mar. ...	1	—	1	3	4	—	1	3	1	—	—	1	1	1
April ...	2	—	3	—	3	—	1	2	1	—	—	1	—	2
May ...	?	—	2	—	2	—	1	1	1	1	—	2	4	—
June ...	?	—	?	—	?	—	?	—	?	—	?	—	2	—
July ...	3	—	?	—	?	—	?	—	2	—	—	2	1	—
Aug. ...	—	—	?	—	?	—	?	—	1	—	—	1	?	—
Sept. ...	—	—	1	1	1	1	—	2	?	—	?	—	?	—
Oct. ...	—	—	1	—	1	—	—	1	?	—	?	—	2	—
Nov. ...	—	—	1	—	2	—	1	1	?	—	?	—	1	—
Dec. ...	—	—	?	—	?	—	?	—	?	—	?	—	?	—
1910.														
Jan. ...	—	—	1	—	1	—	1	—	—	?	1	—	—	?
Feb. ...	—	—	3	—	3	—	2	1	1	—	—	1	1	—
Mar. ...	—	—	?	—	?	—	?	—	1	1	1	1	1	—
April ...	—	—	1	—	1	—	—	1	—	—	?	—	—	—
May ...	—	—	2	—	2	—	—	2	—	—	1	—	—	—
June ...	—	—	—	1	1	—	—	1	—	—	—	—	—	—
Totals ...	32+	9−	31+	16−	37+	7−	14+	30−	11+	2−	5+	10−	18+	4−

? denotes that no examination was made.

It will be seen that the fæces of Mrs. H. (Brentry carrier) were positive on four occasions out of six, during January, February and March, 1909. G., McC., and C. are the three asylum carriers mentioned in Chapter V., pp. 58–60 (A. and J. C. G. Ledingham). Ca. is the intermittent carrier referred to in Chapter X., pp. 84–85. P. is the chronic carrier who suffers from periodical attacks of gall stone colic., Chapter VII., pp. 73–75.

The carriers C. and Ca. are the only two, in whom the negative results exceed the positive.

CHAPTER XIII.

Statistical Data Regarding the Infectivity of Carriers.

This important question will receive more adequate treatment as years go on and data accumulate, and when it has become the established practice for the hygienist to co-operate with the bacteriologist in all enquiries into the origin and spread of enteric fever. Hitherto the available data have concerned only individual instances of infectivity, and, except by a few writers, no attempt has been made to employ these data for statistical purposes. Where, however, bacteriological and epidemiological enquiries have run concurrently over a fairly prolonged period, as in south-west Germany, some exceedingly striking facts have been deduced, which point to the not inconsiderable part played by carriers in the spread of enteric fever. I shall consider in the first place the recent report by Kayser (1909) on the incidence of typhoid fever in the town of Strassburg during the period summer, 1903, to spring, 1907 (nearly four years).

There were 505 cases in all (nearly 141 per annum), with a morbidity of 0·92 per 1,000 of the civil population. (In 1905 the population of Strassburg, excluding the military, was 152,271.) 6·3 per cent. of the cases were due to B. paratyphosus "A" or "B" (1 per cent. "A" and 5·3 per cent. "B").

96·7 per cent. of all the cases were examined bacteriologically, and in 87 per cent. the source of infection is said to have been traced with greater or less definiteness.

Thus—

13 per cent. of the cases came to the town already ill.

26·7 per cent. of the cases are stated to have contracted enteric fever by drinking raw milk, either from houses in which there were typhoid patients, or from dairies in which carriers were employed.

14·6 per cent. were attributed to infection conveyed by water (use of spring or canal water), swallowing of infected water in public baths and at water sports, unclean ice, &c.

2 per cent. were attributed to food-poisoning (Paratyphosus "B.")

11·9 per cent. were attributed to infection derived from contact with typhoid fever patients. Almost all these patients had been atypical or undiagnosed cases.

4·9 per cent. were professional nurses in charge of typhoid cases.

2·2 per cent. were washerwomen in contact with typhoid patients.

9·5 per cent. were attributed to infection derived from carriers (= 48 cases).

Kayser notes that some of these cases due to carrier infection were very severe. He considers that the percentage of cases attributed to infection by carriers is probably too low, and states that his figures include only those cases in which the evidence of carrier infection was absolutely conclusive. From an analysis of his cases he concluded that typhoid fever might be regarded as a disease affecting almost exclusively members of certain trades, particularly those engaged in the preparation of food stuffs, cooks, servants, milk-sellers, and bakers. There was also some special incidence on midwives, apothecaries, and hotel staffs.

During the period indicated, 28 carriers were discovered in the town of Strassburg. Of these nine were chronic carriers, eight excreted the bacilli for some weeks or months, while in the remaining persons, including four children, the bacilli were found only once or twice.

Children were never found to be chronic carriers.

Of the 28 carriers, 11 gave rise to typhoid cases in their neighbourhood, nine probably caused other infections, and eight gave no reason for suspicion.

To summarize, carriers accounted for at least 9·5 per cent. of the total cases of typhoid fever during the period under review.

In the cases due to milk, Kayser includes those in which the milk had been infected by typhoid carriers as well as in other ways, so that the total percentage of cases due to carrier infection (direct or indirect) is probably very much higher than 9·5.

Forster (1908) had already reported the figures relative to the typhoid cases enquired into at his institute in Strassburg during the period January, 1906, to end of June, 1907. In that time 386 cases of enteric were investigated, and 77 of these (or 20 per cent.) were attributed by him to carrier infection.

The following table shows, as regards the total number of cases of typhoid fever, the proportions in which infection was attributed to carriers and to contact with persons suffering from the fever respectively.

—	Total cases of Typhoid Fever.	Infection by Carriers.	Percentage.	Infection by contact with the Sick.	Percentage.
1st Quarter, 1906 ...	37	8	22	3	8
2nd Quarter, 1906 ...	62	18	29	27	44
3rd Quarter, 1906 ...	164	17	10	58	55
4th Quarter, 1906 ...	57	17	30	9	16
1st Quarter, 1907 ...	31	8	26	7	23
2nd Quarter, 1907 ...	35	9	26	13	37
Total	386	77	—	117	—

The percentage of cases due to carriers rose as high as 30 in one quarter, and the average for the 18 months under review worked out at 20.

In Forster's view this percentage is probably too low, as it is possible that many of the small water- or milk-spread infections may have been directly due to some carrier-focus acting only for a very short period.

I come now to consider the statistics for the whole campaign during the four years ending 31st December, 1907. The figures for 1904, 1905 and 1906 are given by Frosch (1907). During that time, as I have already observed, there were 6,708 typhoid fever cases of whom 310 became carriers (144 transitory, 166 chronic). Of these 6,708 infections, 215 were in all probability attributable to carriers, and 61 were attributable to carriers with less probability, *i.e.*, 276 infections or 4·11 per cent. of the total number were ascribed to carriers. The figures for the year 1906 are, however, the only ones available for determining the relative shares of carrier and other agencies in the propagation of typhoid fever.

In 1906 there were 2,080 cases of enteric fever, and in 978 the source was explained (*i.e.*, 47·7 per cent.). Of these 978 cases, 642 were attributed to contact with infectious persons (=28·5 per cent. of the total cases, or 65·14 per cent. of the explained cases). Included in the explained cases are 104 in which it was an open question whether direct or indirect contact was involved. Adding these 104 to the cases definitely attributed to contact we get a total of 746 (= 36·02 per cent. of the total cases, or 76·2 per cent. of the explained cases).

232 infections were attributed to drinking contaminated water, contaminated milk, food-stuffs, &c. (= 11·15 per cent. of total or 23·7 per cent. of explained cases).

1·76 per cent. of the total were imported cases.

Of the 642 contact cases, 49 were due to contact with carriers, = 2·35 per cent. of total cases, or 5·01 per cent. of the explained cases.

Thus about one-fourteenth of all contact infections were due to carriers, while the remaining thirteen-fourteenths were due to ordinary cases of typhoid fever.

Before they were discovered 310 carriers caused 228 infections, = 3·41 per cent. of all cases, while after their discovery and the enforcement of requisite precautions, only 48 infections arose from them, *i.e.*, known carriers were responsible for 0·7 per cent. of all infections.

Klinger (1909) in a more recent report gives the figures up to the end of 1907. The carriers had now mounted to 431 and the total number of infections attributed to them was 351, viz., 51 due to transitory carriers and 300 due to chronic carriers.

These numbers appear small in comparison with the total number of typhoid cases, and, if cursorily considered, might be held to imply that the danger from carrier infection was a relatively small one, but it has to be remembered that though a carrier may infect directly or indirectly one single person, the latter may propagate the infection widely. In fact, endemic prevalences of typhoid fever on a fairly large scale have in the last resort been found to be traceable to a carrier who was responsible only for the first link in the chain.

In the foregoing statistics this view of the matter is not taken into consideration. The carrier is held responsible only for the first link.

For the attainment of complete statistics on the sources of typhoid infection it is necessary that the epidemiologist should not be content to stop his enquiries at the point where he has been able to demonstrate a case of typhoid fever as the immediate source of infection.

These considerations have been insisted upon by Schumacher (1909) in a recent report on endemic typhoid fever at Cröv, in the district of Trier. He comments on the fact that according to Frosch's collected figures only 47·4 per cent of the total cases were explained, and contends that the percentage of cases due to carriers would probably have been found to be much higher had the investigations of the epidemiologist and the bacteriologist been prosecuted with greater insistence. The bacteriological work carried out in connexion with endemic typhoid fever at Cröv, a small typhoid-ridden village, containing about 1,750 inhabitants, was limited only by the reluctance exhibited by a few families to provide material for examination, and even this appears to have been successfully overcome. It is impossible here to detail the whole course of the investigations. The original paper must be consulted and will repay careful study as it is fully provided with charts of the village showing the houses in which cases of typhoid fever occurred in the different years, and those houses in which carriers were resident. I must confine myself here to the actual statistics.

During the period 1903–1908, 45 cases in all occurred. Of this total only 24, *i.e.*, 51·1 per cent had been explained by previous enquiries. As the result of the extensive bacteriological work carried out by Schumacher in the village, practically all the 45 cases received adequate explanation. The following are the figures relating to the different modes of infection :—

Imported cases... ...	6 = 13·4 per cent.	(Previous enquiry			4).
Contact with typhoid cases	20 = 44·4 ,,	(	,,	,,	20).
Cases in which it was doubtful whether the infection arose from carriers or typhoid cases	7 = 15·6 ,,	(	,,	,,	0).
Contact with carriers ...	12 = 26·6 ,,	(	,,	,,	0).

Schumacher says that "the discovery of the chronic carrier gave the key to the explanation of endemic typhoid in Cröv." The large percentage of 26·6 per cent is in striking contrast to that of Frosch (quoted above), and Schumacher suggests that the explanation of the discrepancy is to be found in the following considerations :—

(*a.*) The proportion of explained cases depends on the time that can be spent on the enquiry, on the persistence with which the bacteriological work can be carried out, and finally on the willingness of the people concerned to supply samples for examination. In Cröv these conditions were fully satisfied, whereas the collected data of Frosch were certainly not based on work of such uniform completeness.

(*b.*) It frequently happens that the responsible carrier cannot be detected till some considerable period has elapsed, and when, possibly, the statistics have been already made up.

(*c.*) The statistics only take account of the fact that the first link in a chain of cases was attributable to a particular carrier, whereas the whole chain of cases should really be put to the charge of the carrier if the importance of the latter is to be rated at its true value.

If in the figures for Cröv this latter consideration was taken into account, the number of cases directly due to carriers (viz., 12) would have received an accession of eight indirect cases, giving a total of 20, *i.e.*, 44·4 per cent. These additional eight cases were due to infection from typhoid cases in chains started by carriers.

I close this chapter with the data collected by G. Mayer (1910), regarding endemic typhoid fever in the Bavarian Pfalz. The bacteriological work was carried out apparently in the most complete manner, and to Mayer we are indebted for many important and elemental facts with regard to intermittency and the occurrence of typhoid bacilli in the excreta during the incubation period. In previous chapters many of these data have been referred to. Here I give Mayer's figures relative to the infections caused by carriers :—

—	Number of Infective carriers.	Cases of Typhoid directly due to these.	Site of Infection.					
			In dwellings.	Business.	Bureau.	Social Intercourse.	School.	Shops dealing in food stuffs.
1904 ...	19	63	17	1	2	3	5	4
1905 ...	12	33	12	—	2	—	2	2
1906 ...	25	43	19	—	—	2	3	3
1907 ...	16	21	14	—	—	—	1	1
Total	72	160	62	1	4	5	11	10

During this period there was a total of 495 endemic cases, for 160 or 32·3 per cent. of which carriers were held responsible. As will be seen this figure corresponds fairly closely with that obtained by Schumacher.

With regard to the conditions under which the carriers were infective, the table shows that the great majority of the infections due to carriers arose in the domestic circle, while the school and food shops (bakery, dairy, inns, &c.) constituted a good second. In Mayer's view the unclean hand of the carrier is in most cases the agent which transfers infection to the new host.

CHAPTER XIV.

IMMUNITY QUESTIONS IN CARRIERS.

In previous chapters incidental references have been made to the antibodies demonstrable in the serum of the typhoid-carrier. For diagnostic purposes, at least, the agglutination test has hitherto proved of most service, and I have already discussed the value of this reation and its limitations in Chapter XI.

Here I propose to enter a little more fully into the immunity side of the carrier question in so far as the recorded facts warrant.

In the first place with regard to the agglutinins, about two-thirds to three-quarters of all chronic carriers give a well-marked Widal reaction (titre of 1 in 100 or at least 1 in 50). Some, indeed, have been found to exhibit a very high agglutinin content (up to 1 in 2,000 or higher), but such high titres are generally associated with an intercurrent acute infection or auto-infection such as cholecystitis.

Owing in great measure to the reluctance of healthy carriers to furnish specimens of serum and the insufficient facilities for work of this nature in institutions where carriers are isolated, our knowledge of the variations in the agglutinin-content of carrier-sera over a long period, is very deficient. An investigation of this matter might yield results of great value.

With regard to the transitory carrier who has never had any obvious symptoms of typhoid fever or who has so far not developed symptoms, definite statement as to the course of the agglutinins cannot yet be made. We know that typhoid bacilli may be excreted in the fæces and may even be circulating in the blood-stream for some considerable time before the agglutinins make their appearance in the serum. This fact may explain the recorded absence of a Widal reaction in a certain proportion of transitory carriers. On the other hand, a development of agglutinins has been observed to occur in the so-called "symptomless" cases of typhoid fever (Lentz, Scheller, already cited), who are found to discharge bacilli for very short periods. It is important to remember these points in those cases in which the physician has a positive reaction reported to him, which however, does not appear to be associated with any definite symptoms of the disease. Such cases undoubtedly occur and may give rise to considerable scepticism as to the value of the Widal test. For the satisfaction of the physician and the bacteriologist, an attempt should always be made to examine the excreta in view of the possibility of such cases being really temporary carriers.

I have already discussed certain other limitations of the Widal test in carriers, particularly its not uncommon occurrence in cases of miliary tuberculosis which do not exhibit typhoidal lesions at autopsy. In this type of case the excreta should always be bacteriologically examined, especially in children, in whom notoriously the course of typhoid may be atypical. It would take too much space to enter into the question of positive Widal results either in typhoid-carriers who also harbour organisms of the Gaertner-paratyphoid group or in persons infected with members of this group only. The presence of a co-agglutinin for B. typhosus in the serum of food-poisoning cases is now well established, though no satisfactory explanation has hitherto been given. In this monograph I have purposely avoided the discussion of mixed infections (typhoid with paratyphoid, &c.), in view of the present unsettled state of opinion with regard to the etiological importance of this latter group.

One other point relating to the agglutinins in carrier-sera has to be observed. The serum of the carrier may agglutinate with ease the bacilli of a laboratory strain, but may have little or no influence on the homologous strain. It must be noted, however, that the reaction with the homologous strain may occur late, the velocity of reaction being much slower than with the stock strain.

Ledingham (1908) recorded the results with two carrier-sera tested, both with the stock strain and with the homologous and other carrier strains. One of them gave a marked reaction with the laboratory strain only, but with the homologous strain and another carrier strain there was merely a trace of agglutination after nine hours. On the other hand another carrier serum was able to agglutinate with equal ease all three strains. Kennedy (1910) records similar results.

In those carriers in which no agglutination of the homologous strain takes place, one cannot assume that the serum contains no suitable receptors of any kind for this strain, as we know that the serum may still possess a high opsonic or tropic content towards the homologous bacillus. Further, as we shall see, there may be no agglutinins in the serum either for stock or homologous strains and yet complement-deviating bodies may be demonstrable.

The influence of vaccination (with typhoid vaccines) on the agglutinin-content of carrier-sera varies greatly. In one case (Thomson & Ledingham (1910)) the titre rose from less than 1 in 20 to 1 in 800 towards the stock strain, and to 1 in 400 towards the homologous strain. Recently, however, I have had a case in which the agglutinins remained stationary even after a course of vaccination with the patient's own strain. Kennedy (1910) has recently published charts showing in two cases either no change or only a very insignificant rise in the agglutinins, and in one case a stationary titre followed by a marked rise to 1 in 300 after the use of a specific vaccine.

Bactericidal Substances.

Observations on the bactericidal power of the serum of carriers are few, and these go to show that little or no variation from the normal standard occurs.

On two occasions (Ledingham (1908)) I have tested the sera of three carriers against the homologous and other strains (in vitro tests). The sera were inactivated and complemented with fresh guinea-pig serum. Some increase in the bactericidal power of the serum was found over that of the control serum, but the differences appeared to depend largely on variations in the resistance of the strains employed.

A sufficient quantity of serum for experiments of this nature is not readily available. Niepratschk (*vide* Chapter VI.) tested by the Pfeiffer method the bactericidal power of the serum of his urinary carrier. The virulence of the carrier strain was raised by passage through the guinea-pig from one-quarter of a whole agar-culture to one-quarter of a loop (= minimal lethal dose for guinea-pig of 250 gms. by intraperitoneal inoculation). With this strain and the carrier serum he made the following experiment :—

Guinea-Pig.	Result.
A. $\frac{1}{4}$ loop + 1 c.c. of 1 in 200 dilution of serum.	Death after 24 hours.
B. $\frac{1}{4}$ „ + 1 „ 1 „ 100 „ „	„ 24 „
C. $\frac{1}{4}$ „ + 1 „ 1 „ 50 „ „	„ 24 „
D. $\frac{1}{4}$ „ + 1 „ 1 „ 25 „ „	Lived.
E. $\frac{1}{4}$ „ + 1 „ 1 „ 10 „ „	„
F. $\frac{1}{4}$ „ without serum	Death.

The bactericidal titre, therefore, lay between ·02 and ·04 and was thus very low.

Opsonic Substances.

Ledingham (1908) found a very marked increase in the opsonic content of carrier-sera towards homologous strains, indices of 4 to 9 being obtained. Further, this opsonic substance disappeared almost completely on inactivation of the serum, but was readily restored on complementing with fresh normal serum, which of itself had little opsonic value. An opsonic amboceptor was thus demonstrated in carrier-sera.

Gaehtgens (1909) examined 16 carrier-sera and found in all except one (an intermittent case) a marked increase in the opsonic content. He also tested the sera of a number of persons who had had enteric at varying periods previously, but who were not carriers. An increase of opsonic power could be demonstrated only in those whose attack dated three or four months back.

In a recent communication Kennedy (1910) has reported a considerable increase in the opsonic content of carrier-sera. During vaccination a further steady rise takes place, which may not be accompanied by any definite increase in the agglutinin content.

Hamilton (1910) has also found abnormal opsonic indices in typhoid-carriers and recommends the opsonic tests in the preliminary search for carriers.

Complement-Deviating Substances.

Schöne (1908) investigated this question in three carriers and in the serum of two of these he was able to demonstrate quite definitely the presence of complement fixing-bodies. One of my colleagues—Henderson-Smith—(experiments not yet published) has also been successful in demonstrating these substances in the sera of two untreated typhoid-carriers, both of whom gave completely negative Widal reactions.

In concluding this chapter one feels compelled to admit that specific antibodies for the typhoid bacillus do pass over into the general blood stream in typhoid-carriers, although the vegetative foci may be considered to be in great measure shut off from or even, as some would suggest, entirely outside, the influence of the general circulation. The "local immunity" theory of Wassermann and Citron (1905) can only, therefore, afford a partial explanation of the prolonged vegetation of the typhoid bacillus in the gall-bladder and biliary tracts. Much work yet remains to be done on the immunity aspect of the carrier question.

CHAPTER XV.

VARIOUS MEASURES EITHER EMPLOYED OR SUGGESTED TO DIMINISH THE SPREAD OF INFECTION BY CARRIERS.

In this concluding chapter I propose to discuss briefly the various precautions which have been adopted or recommended by sanitary authorities and others in order to reduce as far as possible the risk of infection from carriers. Pending the discovery of some efficient mode of therapy, all attempts to cope with this danger by sanitary measures are worthy of the most serious consideration at the present time. Obviously the first step is the adoption of some form of routine bacteriological examination during convalescence, and in previous chapters I have sufficiently alluded to the practice in vogue at the typhoid stations in Germany and elsewhere, and to the drawbacks from which practically every stereotyped routine must suffer, owing to intermittency, imperfection of bacteriological method, or both. Still there is no doubt that much good can be accomplished by the early recognition of carriers, and experience has shown that the longer the duration of bacteriological supervision the less is the risk of overlooking the intermittent carrier. Monthly examinations of the excreta of all convalescents for a period of one year would probably suffice, but in view of the great practical difficulties that such a scheme would involve, I do not propose to discuss it here.

Otto Mayer (1909) has suggested in a recent paper the foundation of Convalescent Homes for recovered typhoid cases. In such homes the convalescent who is still excreting the specific bacilli on discharge from hospital would be retained. By suitable treatment, dietetic and otherwise, by gymnastic exercises, &c., efforts would be made to encourage the physiological excretion of typhoid bacilli until possibly complete cessation resulted. Whereas many convalescents are compelled to resume their active duties at a far too early stage, when consequently the natural recuperative powers of the organism do not get an opportunity of throwing off an infection that is tending to become indolent, in the Convalescent Home every opportunity would be afforded to the bactericidal and antitoxic powers of the organism, of exercising their full effect. Full and accurate statistics would be kept regarding the duration and severity of the disease, the diet employed in convalescence and in the home, the muscular power of the patient and the bacteriological results. Such statistics would later prove of great value in deciding the influence exerted by the treatment during convalescence on the carrier-output.

There is no doubt that such institutions would prove of service in many ways. The patients that would ultimately have to be discharged in a potentially infective condition would be accurately instructed as to the precautions they should take on their return to civil life. This of itself would be a great gain, as the difficulty of impressing on unintelligent carriers the necessity of taking strict precautions as to disinfection has proved a serious stumbling block.

In institutions such as lunatic asylums the problem of dealing with chronic carriers who are also chronic lunatics is solved by the simple and successful expedient of isolation (*see* Chapter V.). This measure, however, may prove an expensive one, especially in the case of small institutions, as it may involve the upkeep of an isolation block with special attendants for a period coincident with the life of the patient.

Grimme (1908) has stated that prolonged isolation is unfavourable to the patient, as little or no intercourse is possible and the patients themselves do not see the necessity for the detention. This may certainly be true in some cases, but so far as the asylum cases that I have had under bacteriological supervision for two and a-half years are concerned, I am informed that isolation has had no prejudicial effect. It may be otherwise with acute lunatics who are carriers.

Every new patient, on entrance into the asylum, should be examined bacteriologically.

In the Army, measures have already been taken for dealing with known carriers. If, after a course of therapeutic treatment the carrier-condition persists, the soldier is discharged from service with or without a pension according as he agrees or not to the notification on his discharge-sheet, that he is a chronic typhoid-carrier. Drastic steps have also been taken to prevent the employment of regimental cooks who may happen to be carriers. The following "Standing Orders" for the Military Medical Services in India, 1908, I quote from a recent paper by Carmichael (1910), who enters a plea for the registration of typhoid-carriers in the army :—

> "No man who has recently suffered from enteric fever should be so employed (whose duties entail the handling of food intended for British troops, in kitchens, Officers' and N.C.O.'s messes, regimental and other institutions and clubs, government and regimental dairies, bakeries, mineral water factories, &c.) until it has been placed beyond all doubt, by repeated bacteriological examinations of fæces and urine that he no longer harbours the *Bac. typhosus*. In cases where means for carrying out such examinations are not available these men must not be employed."

Carmichael would propose the addition of this order to the Home Regulations with the wording "who has recently" altered to "who has ever."

At the Fleetwood station, Carmichael allows no man who is known to have suffered from enteric fever to be employed either in the kitchen or as orderly man. Certainly where facilities are not available for determining whether men who have had enteric fever, are carriers or not, this plan may be usefully adopted, but it does not exclude the risk of employing men whose primary infection may have been so slight as to remain unrecognised and who may quite possibly be chronic carriers.

Semple and Greig (1908), in the Indian Army advise the careful bacteriological examination of all men (Europeans and natives) before permitting them to be employed in connexion with the preparation and distribution of articles of food and drink.

In the civil population the problem of dealing effectively with carriers revealed in the course of enquiries into occurrences of enteric fever presents difficulties which only the future can solve. At present much good is being done in south-west Germany and elsewhere by retaining all carriers under bacteriological supervision, by giving suitable instruction with regard to disinfection of their excreta and their persons (particularly their hands), and by oral and verbal advice as to the occupations they should avoid. A useful series of "Standing Orders" employed in the Pfalz stations, accompanies a recent paper by Mayer, G. (1910). Of one of these, dealing with typhoid-carriers and convalescents, I give a translation. It has been in force since 1904.

1. Typhoid carriers and clinically cured cases are retained under bacteriological supervision until a bacteriological cure results.

2. So long as they discharge bacilli, the official disinfecters carry out or supervise the continual disinfection of privies used by them with milk of lime.

3. In the case of school children, workers in factories and such like, teachers, officials in state and private bureaus, these may carry on their respective duties provided that the schools, factories, &c., are provided with suitable wash-basins and soap. On commencing duty and on every occasion when the privy or urinal is visited, the hands must be cleansed.

4. In dwellings the disinfection of privies and urinals is systematically carried out by the official disinfecters (with milk of lime).

5. Typhoid carriers are to be restrained from the preparation and sale of food stuffs.

Instructive pamphlets are also issued to every newly-discovered carrier. The following is a free translation of that issued to carriers by the typhoid station at Strassburg (quoted from Gaehtgens (1910)).

VERHALTUNGS-MASSREGELN FÜR TYPHUSBAZILLENTRÄGER.

(*Instructions for Typhoid-carriers.*)

1. Typhoid fever in its severest form runs the course of a nervous fever. In its slighter forms it is accompanied by diarrhœal symptoms, but frequently the symptoms of the disease are scarcely noticeable.

It is caused by a specific bacillus—the typhoid bacillus.

2. The typhoid-patient harbours the typhoid-bacilli in his body, and discharges them during the disease with the stools and urine.

3. Some persons may continue to discharge typhoid-bacilli in the fæces for months or years after convalescence, or even although there has been no obvious primary attack. These persons are called typhoid-carriers.

4. Typhoid-carriers suffer as a rule from gall-bladder troubles, which give rise to transitory colic, jaundice and gall-stones, but frequently these symptoms are not present.

5. Carriers may infect other persons with typhoid fever just as typhoid-patients do.

6. Infection occurs most frequently by the hands, which are readily contaminated with typhoid bacilli during defæcation and urination. Infection may also be spread by soiled body- and bed-linen.

7. The bacilli get transferred from the unclean hands to other persons either by direct contact or by indirect contact, *e.g.*, with food-stuffs and various objects used by other persons.

8. It is possible for typhoid-carriers to reinfect themselves. In such cases one may expect an aggravation of the already existing gall-bladder troubles.

9. Carriers may avoid infecting other persons or themselves by the following precautions :—

(*a.*) The carrier must always exercise the greatest cleanliness.
(*b.*) Hands should be thoroughly washed on getting up in the morning, before every meal, and before touching food or drink (especially milk).
(*c.*) The carrier must, after using the water-closet, cleanse the hands thoroughly with soap and water or with a disinfectant. There should always be a sufficient quantity of closet-paper in the water-closet.
(*d.*) The carrier must have his own towel, which no one else may use.
(*e.*) The carrier's dirty body- and bed-linen should, before washing, be dipped in cresol-water (two tablespoonfuls of cresol-soap solution to one litre of water).
(*f.*) The carrier should, as far as possible, avoid taking part in the actual preparation of food or in its sale.

10. It is to the carrier's own interest that he or she should send, about once a month, a specimen of the stools and of the urine to the Bacteriological Institute at Strassburg in order that it may be determined whether the excreta still contain typhoid bacilli. The examination is done gratis. Suitable vessels for the reception of excreta are sent post-free from the Institute.

11. The Institute is very pleased to give advice regarding the disinfectant to be used by carriers.

12. The precautions advised above are absolutely indispensable, and it is morally incumbent on carriers to adhere to them in order to avoid infecting other people.

Davies and Walker-Hall (1908), of Bristol, have also issued a pamphlet containing instructions which, they suggest, should be given to typhoid convalescents. These are reproduced here :—

SUGGESTED INSTRUCTIONS TO TYPHOID CONVALESCENTS.

(*To be handed to Patients when Discharged from Hospital.*)

Typhoid fever is caused by a germ (the typhoid bacillus) which enter the body in food or drink or swallowed dust. When the convalescent stage is reached, the germ may remain in the intestines and cause relapses of the disease. Sometimes the germ remains permanently in the intestines, and may cause a return of the disease many years after the first attack. The germ may also induce the formation of gall-stones. Thus it is evident that the contents of the intestine of the typhoid convalescent are infective, and that the convalescent may be the unconscious means of transmitting the disease to his, or her, own family or friends. Cooks, male or female, dairymen or dairywomen, milk-vendors, greengrocers, butchers, fishmongers, provision dealers, workers in cocoa, chocolate, or confectionery, waiters and waitresses, and the mother who prepares food for the family, form a class of convalescents in which the opportunities of transmission of the disease are very frequent. The following provisional rules are suggested with a view to diminish the risk of infection :—

1. The hands and nails should be thoroughly washed, first in disinfectant solution, then in soap and water, and well rinsed before touching any foodstuffs, especially milk.
2. After the bowels are opened or the bladder is emptied, the hands and nails should be at once disinfected and washed.
3. Strong disinfecting solution should be poured over the stools, &c., before the plug of the water-closet is used or the lid of the dry closet is shut down.
4. When the motions are loose the risk of infection is increased and additional care should be taken.
5. The convalescent should periodically visit the medical attendant, so that the blood, &c., may be tested, and measures may be taken to control the action of the germs. As there is generally no need to deal with the drains, or to disinfect rooms or houses for these special cases, no upset of the domestic arrangements is necessary.
6. In the household where a patient is convalescing from typhoid fever, the additional precautions of boiling all the milk or water used for drinking or in the preparation of food should be undertaken, and uncooked foods should be avoided.

There is no doubt that much good has already been accomplished by instructing typhoid-carriers in proper methods of disinfection, and it is on the disinfection of the hands that chief stress must be laid. Gaehtgens (1910) has performed an interesting series of experiments designed to determine the most efficient and at the same time the least irksome method of cleansing the hands of typhoid-carriers. He smeared the hands and fingers with fæces containing typhoid bacilli, and determined the relative disinfectant values of mechanical washing, with and without disinfectants, and followed or not followed by thorough drying. He found that washing with soap and water alone, without subsequent drying of the hands, caused a marked fall in the number of B. coli and B. typhosus, especially of the former. If, however, the hands were dried thoroughly after removal of the soap in running water, subsequent cultivation frequently showed a complete disappearance of B. coli and B. typhosus.

For those carriers, therefore, who are not specially engaged in handling food-stuffs, the mechanical cleansing of the hands with soap and water and subsequent drying affords a tolerably complete protection. For those carriers on the other hand, whose occupation entails the handling of such material, it is advisable to employ an antiseptic after the ordinary washing and drying process in order to ensure complete removal of any typhoid germs still clinging to the hands. For this purpose Gaehtgens found that the most satisfactory disinfectant was alcohol either in the form of eau-de-cologne or spirits of wine.

APPENDIX.

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DYSENTERY CARRIERS.

Being a Paper read in the Section of Bacteriology at the Annual Meeting of the British Medical Association, London, July, 1910.

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EPIDEMICS of bacillary dysentery in this country are at the present time limited almost entirely to the precincts of lunatic asylums and such institutions, although sporadic cases, often unrecognized, occur also among the general population. The nature of these endemics is of a type only too well known to the officers in charge. Infection is everywhere, and most of the inmates become attacked sooner or later. There appears, however, to be a species of natural immunity which protects the robust—members of the staff, for example—but this may be overcome by a big dose of infection.

Epidemics may be due to the presence of either the Shiga or the Flexner bacillus. At times, moreover, the two types may occur side by side in one epidemic. Outbreaks due to infection with the Y bacillus occur in Germany, characterized by a relatively high proportion of chronic cases The particular epidemic that I have studied, with which I propose to deal presently, owes its origin and maintenance to the Flexner bacillus, but in a small proportion of cases, indistinguishable by clinical means from the majority, other organisms, such as *B. pyocyaneus*, Morgan's bacillus No. 1, *B. proteus vulgaris* have been found alone—instances, perhaps, of secondary infections.

The nature of dysentery outbreaks is such as to suggest that the disease is propagated by contagion, direct or indirect, from person to person They lack the explosive character of water or milk epidemics The course of the outbreak bears a strong resemblance to that of other diseases in which carriers have been shown to constitute a prominent etiological factor. Particular incidents in the history of a number of dysentery epidemics supply evidence which indicates that here, too, carriers may be of great importance

Carriers, in the widest sense, may be distributed into two great classes The first class consists of healthy persons who have never suffered from the disease, and show no signs of ill health, but nevertheless harbour and scatter the specific organism. Those, on the other hand, who make up the second clsss, are persons who have had the disease, and are thereafter unable completely to eliminate infection. Their after-history is chequered by minor disturbances of health, and by intermittent liberations of the specific bacterium.

Cases of the first type, in the instance of dysentery, are rare, and probably unimportant. Conradi recovered the bacillus from five healthy children, all of whom had, however, been living in the midst of infected adults. Two similar cases, also in children, are reported by Collins. Otto Mayer, while investigating an epidemic of pseudo-dysentery, found a relatively larger number of healthy carriers, but of these persons at least one developed the disease after isolation. I have not been able to find a single case of this type. As regards convalescent carriers, I am unwilling to dogmatize prematurely, but my experience appears to go far in confirming the view that, as agents in the spread of dysentery, the importance of this class cannot well be over-estimated.

During the past half year I have had the opportunity of studying an epidemic occurring at a large lunatic asylum in the Midlands, and I am glad to have this opportunity of expressing my thanks to the medical superintendent for placing the material at my disposal. As the disease has been prevalent for a number of years, it has been possible to obtain specimens from patients at all periods—some during or shortly after attack, others after widely varying periods of freedom from disease. A rise in the number of cases took place during April, and in consequence it has been possible to make a particular study of these persons during early convalescence.

From 120 inmates I have received about 200 specimens of faeces to examine bacteriologically. Concurrently, agglutination tests were performed upon a rather smaller number of serums from the same persons. After a short account of the results of this work I propose to attempt a classification of cases and an estimation of the number of potential carriers.

The methods pursued have been of a simple and stereotyped nature. In dealing with faeces, a loopful was plated upon MacConkey's lactose neutral-red bile-salt agar. From these plates, after incubation, suspected colourless colonies were picked off into mannite peptone water. Cultivations that showed an acid reaction in this medium were tested for a further series of sugar-fermentation reactions. Complete identity was established by testing the degree of agglutinability of organisms with a Flexner-rabbit serum of high potency.

In order to select a suitable portion of a sample for plating it was necessary in some cases to spread the material in a sterile dish. Mucus was taken if possible, as this is the most usual habitat of the bacillus. Second or third platings were performed before a negative result was recorded, but even so, negative results are far less certain than positive. A minute portion only of a small sample of a single stool is examined, and thus much territory must be left unexplored.

Twenty-eight specimens from acute cases gave 13 positive results Among the remaining 15 negative samples there were 7 from which some other pathogenic form was isolated, which may have masked or replaced the primary infection. *B pyocyaneus*, for example, was obtained three times. As a rule, the Flexner bacillus was isolated readily from typical acute dysenteric stools containing mucus and blood. With disappearance of blood and diminution of mucus the proportion of positive findings diminished greatly. Almost invariably the bacillus could no longer be recovered from stools after complete return to the normal faecal type. The table below shows the condition of affairs in the April and May cases where it has been possible to make repeated observations during early convalescence.

	First Week.	Second Week.	Third Week	Fourth Week	Second Month.	Third Month.
Percentage of samples from which Flexner's bacillus was recovered	67	33	25	16	20	0
Percentage of cases suffering from definite clinical attack	100	23	8	4	1	0

These figures show that clinical convalescence is in advance of that determined by bacteriological examination

The difficulty of proving a complete negative by these bacteriological methods gives an added importance to agglutination results.

The strain of Flexner bacillus used for this purpose was one isolated from the earliest positive case examined. The dilutions of serum investigated ranged from 1 in 25 to 1 in 400. Microscopic methods were employed throughout. Complete clumping in dilution of 1 in 100 was regarded as positive, but lower dilutions were in general disregarded.

Twenty-five serums from acute cases gave seventeen positive results. Four of the remaining eight negative samples were obtained at the very commencement of the attack—that is to say, before the development of specific agglutinins.

	Positive Agglutinations.	Negative Agglutinations.
During attack	17	8
After 6 months	5	6
6 months to 1 year	7	6
1 to 3 years	12	12
3 to 5 years...	4	5
5 to 8 years	1	4

This table shows that while in many cases agglutination no longer occurs a few months after an attack, there is a very large number in which, even after a lapse of years, the serum retains the power of clumping in high dilutions.

Examination of serums obtained from persons during quiescent seasons showed that, unlike typhoid, there is no definite time limit for the disappearance of specific agglutinins after termination of an attack.

Unsupported by other evidence, persistence of agglutination must not be thought invariably to connote persistence of infection. The problem is more complex than this. On the other hand, in the presence of such residual agglutinations it is difficult to prove that these cases no longer contain an infective focus. Moreover, when the history of such persons is chequered by periodic disturbances of health, amounting often to definite relapses, the presumptive evidence in favour of the existence of such a focus becomes very strong. This gives such an importance to residual cases that it becomes necessary, before proceeding to classification, to consider them in detail.

A review of the clinical features of the whole series shows that persons who recover completely from acute dysentery possess considerable powers of resistance to further infections In the rare cases where such persons yield to a fresh infection from without, the second attack is acute and pursues a similar course to the primary attack.

In relapsing cases, in which it is supposed that further infections come from within, the history is different. That convalescence from the primary acute illness is incomplete is shown first, by the persistence of a high agglutination figure; and secondly, by the continued presence in the faeces of mucus, albeit in greatly varying degree. Further attacks are subacute, and may possibly last for no longer than a single day, but sometimes they may be intractable and continue for relatively long periods. Attacks may be determined by circumstances which lower the general resistance. Cold, bad weather, and particularly unsound food, are the most obvious exciting factors. The series contains records of twenty-four occasions upon which unsuitable diet provoked a relapse. In rare cases returns of the disease become frequent and protracted, and the illness assumes a chronic form. But this is rare in the case of Flexner dysentery.

It is not yet possible to dogmatize as to the site of such persistent infective foci, but in analogous cases of infection with the Y bacillus—where, however, the disease is of a more chronic type—rectal ulcers have been seen with the rectoscope. If the focus be in the intestinal wall, it is probable that bacilli are in fact constantly being passed out. During quiescence, when the motions are well formed and the amount of bacteria very small, the danger of infecting others is negligible, even apart from any possibility of diminution in virulence or toxicity—a point not yet worked out. But at times, apart from recognized attacks, there may occur minor disturbances—manifested, it may be, by a single stool rich in mucus—which may escape the notice of both patient and attendant, and consequently become a fertile source of infection. The system of classification depends upon the completeness of convalescence after the primary attack. Recent cases which have not been under observation for a sufficiently long period are not included in the following table:

	Male.	Female.	Total.	Percentages.
1. Complete recovery	20	23	43	53.7
2. Single attack, but agglutination remains high; stools usually contain a trace of mucus	9	7	16	20
3. Relapsing cases	4	15	19 } 21	26.2
4. Chronic cases	—	2	2	

The first group contains persons who after the primary attack return to normal health and are for a season immune against fresh invasions. Members of asylum staffs and the more robust inmates come under this heading.

The second group is made up of those cases whose complete recovery is only discounted by the continuance of agglutination, and by the presence of small quantities of mucus in the faeces They do not relapse. Possibly they harbour infection somewhere, but further evidence is required to secure conviction. Administration of a simple purgative might perhaps determine the elimination of bacteria.

Types of Cases.

Group II.—G. R., male, aged 55. Attack April 11th, 1905, lasting three days. Result of examination January 25th, 1910: Faeces negative; agglutination positive ($\frac{1}{400}$).

Group III.—F. H., female, aged 24. Five attacks between June 29th, 1906, and January 9th, 1910. Result of examination February 3rd, 1910: Faeces positive; agglutination positive.

Group III.—J. A S., female, aged 37. Four attacks between September 16th, 1903, and April 2nd, 1909; two attacks provoked by food. Result of examination February 1st, 1910: Faeces negative; agglutination positive ($\frac{1}{400}$).

The third group contains relapsing and chronic cases, whose nature has already been described To define the position of these three groups with regard to the question of potential carriage, the language of Scots law may be borrowed and verdicts of "not guilty," "not proven," and "guilty" be returned. The figures in the table show that, if this view be accepted, 26 per cent. of these cases must be regarded as possible transmitters of contagion. These considerations emphasize above all things the great need for care during and immediately after the primary attack. The first step towards the prevention of an epidemic must be one that obviates the development of a class of incomplete convalescents. To this end no method of treatment, local or general, should be omitted. During early convalescence the same care should be taken as during the attack. The consistence of the stools should be watched, and Widal's tests be performed from time to time. Until the results of these observations be satisfactory, these persons must not be allowed to mingle with their healthy fellows. Persons that have already come under the heading of incomplete convalescence must be strictly isolated, while means should be taken to discover, if possible, and eliminate the focus of infection. Members of the second group should be domiciled in a separate block, if possible, and it is superfluous to add that no laundry or kitchen work should be assigned to any such suspected persons.

The conclusions, put very briefly, are:

1. That healthy carriers are very rare, and of no importance.

2. That the actual carriers are to be found among the incomplete convalescents, which form a high proportion of the cases.

3. That in combating an epidemic it is necessary to reduce as far as possible the number of such cases, and to isolate very strictly those that are already of this type.

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THE PHYSIOLOGICAL MECHANISM OF ANAPHYLACTIC SHOCK.—A PRELIMINARY COMMUNICATION.[1]

A SUMMARY.

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The development of the science of immunity has thus far [275] been mainly in the hands of investigators, interested in the bacteriological, chemical and commercial aspects of the subject. The study of serum phenomena in their broader relationships, particularly the determination of the deeper-seated physiological reactions, of which the serum changes are conceivably but superficial indices, are parts of the subject thus far largely neglected. Yet it is probable that it is mainly through the development of this broader sero-physiology that are to come the important practical results of the future.

As an introduction to a series of such sero-physiological

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It is my privilege to acknowledge my indebtedness in this work to the important suggestions of Professor Starling, and to express my thanks to his associates in the Institute of Physiology, for their cordial encouragement and criticism, that have lent so much to the pleasure of my stay in his laboratory, particularly to the many kindnesses of Associate Professor Bayliss. My thanks are also due to Professor Cushny, for his kind personal interest and suggestions. I am furthermore indebted to Dr. Martin, director of the Lister Institute of Preventive Medicine, for the hospitality of his laboratory and for numerous important criticisms, and to Mr. Twort, director of the Brown Institution, for the use of his kennels.

[275] studies, I have chosen to investigate certain features of the physiology of anaphylaxis. The assumed importance of anaphylaxis, in the development of the theory of immunity, and the ease with which this phenomenon is studied by the simpler physiological methods, have influenced this selection.

Certain phases of anaphylactic physiology have already been studied. Thus Besredka, Gay, and others have applied sera directly to the central nervous system and nerve trunks; Biedl and Kraus, Pierce and Eisenbrey, and others have investigated certain of its vasomotor features; while Auer and Lewis have called attention to important respiratory manifestations. It is the aim of the present paper to carry the physiology of anaphylaxis, back of the phenomena studied by these investigators, to what I believe to be the primary anaphylactic mechanism, a reaction of the tissues and organs concerned in protein digestion and assimilation.

The work herein reported is part of a rather ambitious anaphylactic study, involving the use of cats, dogs, rabbits, guinea pigs, frogs, turtles, and other forms. Dogs were found most suitable for the phases of the work dealt with in this paper, and form the basis for this preliminary communication.

These dogs were sensitized by an initial subcutaneous injection of horse serum, 1 cc. to 2 cc. per kilo. of body weight, and were studied about 18 days later, under morphine-chloroform-ether anæsthesia. The anaphylactic shock was induced in these animals by an intravenous injection of dilute horse serum, the most frequently employed dose being 10 cc. of 25 per cent serum.

As an introduction to this work, numerous respiratory, blood-pressure, and plethysmographic tracings were made, nerve trunks were sectioned, electrical reactions studied, as well as reactions to therapeutic agents. This part of the work confirmed, in the main, the results of previous investigators, though my data led me to look upon the anaphylactic shock in dogs as physiologically more complex than Biedl and Kraus
[276] have assumed. This part of the work, however, was interrupted, as soon as it was discovered that all of the phenomena

with which I was dealing were apparently but secondary and tertiary manifestations of a deeper-seated primary reaction. [276]

This discovery came from attempts to locate the anaphylactic material in the body. It was shown that a cross circulation, continued for about five minutes, between an anaphylactic dog and a normal dog, usually confers immediate anaphylactic properties on the normal animal.

This proves that part, at least, of the material, formed in response to the initial serum injection, responsible for the subsequent anaphylactic condition, exists in the circulating medium. That the circulating fluid, however, is not the only store of this material, is shown by experiments, in which the blood of an anaphylactic dog is washed out and replaced by normal blood. Although such an animal is left with but negligible traces of its original circulating anaphylactin, nevertheless it may now react strongly to a serum injection.

The amount of normal blood used in these experiments was so great, and the washing-out process was extended over so long a period of time, that it is believed, not only that the circulating anaphylactin was completely washed out, but that the anaphylactin existing in the tissue spaces was also removed. It would, therefore, be logical to look upon the non-circulating anaphylactin as probably existing in combination with fixed body cells, presumably held in those cells in which it was originally manufactured.

In many cases, however, a simple cross circulation between two dogs fails to confer anaphylactic properties on the normal animal, although the sensitized animal may be shown, by a subsequent test, to be highly anaphylactic. This could be very easily accounted for by assuming the absence of a sufficient amount of circulating anaphylactin in the sensitized animal to transfer the hypersensitive condition, were it not for the fact that occasionally a dog which in itself has apparently failed to develop anaphylactic properties after the initial injection, since it gives no reaction on a subsequent serum test, is nevertheless, capable of conferring strong, immediate anaphylactic properties on a normal dog, by such simple blood transference.

These facts have led me to assume that the anaphylactin

[276] was not the only factor determining the shock. According to this assumption, the reaction would take place only when the anaphylactin is supplemented in its action by certain necessary conditions in the reacting tissues.

This view is strengthened by numerous transfusion experiments. Thus an anaphylactic dog, after recovering from the shock of one serum injection, is for some time insusceptible to a second serum dose. Nevertheless the blood of this animal, transferred to a normal dog, may confer on the latter immediately anaphylactic properties. Furthermore a dog rendered anaphylactic by transferring to it the blood of a sensitized animal, will, after reacting to one serum injection, remain insusceptible to a second serum dose, even after its blood and the waste products of the reaction have been thoroughly washed out and replaced by a fresh supply of anaphylactic blood. The reacting tissues have apparently become refractory.

To determine which tissues cooperate with the anaphylactin in producing the shock, a large amount of work was planned with isolated organs. The problem, however, proved easier of solution than anticipated. If temporary ligatures are placed about the aorta and vena cava, above the diaphragm, the upper half of the anaphylactic body will not react to a serum injection. The supra-diaphragmatic tissues and organs, therefore, are not primarily concerned in anaphylaxis. This rules out at once, the central and peripheral nervous systems and nerve endings, the cardio-vascular and pulmonary mechanisms, the connective tissue, lymphatic tissue, smooth and striped muscle as being part of the primary mechanism.

On now releasing these ligatures, allowing the serum to pass below the diaphragm, a typical shock may develop. The primary mechanism in the dog is therefore sub-diaphragmatic, and is presumably to be sought among the sub-diaphragmatic tissues not represented above the diaphragm. This confines our search at once to the abdominal viscera.

A removal of the intestines, from the pylorus to the rectum, with the attached pancreas, does not prevent the shock. A further removal of the stomach, kidneys, spleen, adrenals, ovaries, and uterus, all of which can be done in dogs without [276] interfering with the hepatic artery, does not prevent it. We are, therefore, forced to conclude that the essential primary organ, is the only remaining abdominal viscus, the liver.

Confirmatory proof of the hepatic origin of the anaphylactic shock is, however, difficult. Attempted removal of the liver usually throws dogs into such profound surgical shock, as to render them useless for subsequent anaphylactic tests. Methods were, therefore, sought to ligate off the liver, without interfering with the circulation in other organs. As a preliminary to such attempts, it was shown that rendering dog's blood non-coagulable by defibrination, or by the use of leach-extract (hirudin), does not destroy the animal's anaphylactic properties.

If the intestine is removed, so as to eliminate the portal circulation, and if, after the injection of hirudin, a T-canula is now placed in the abdominal vena cava leading to the external jugular, the liver can be readily excluded by simple ligatures. Anaphylactic dogs, thus partially eviscerated, do not react to a serum injection. On releasing the ligatures, allowing the blood once more to pass through the liver, a shock may develop.

This operation, although it establishes the hepatic origin of the anaphylactic reaction, does not prove that the liver is the only primary organ concerned. Such proof could only come from excluding the liver from animals in which the intestine is intact. By placing an additional canula in the portal vein, carrying the portal blood also to the external jugular, this purely liver exclusion can be effected.

Of the six successful positive experiments of this nature, four animals gave no shock with the ligatures closed, but developed reactions on their subsequent release. Two of the [277] animals, however, gave slight shocks with the liver excluded. We are, therefore, forced to conclude that, in hirudinized animals at least, a non-hepatic shock is possible, from which we must look upon the intestine with the attached pancreas, as constituting a second, primary anaphylactic mechanism.

That the liver and intestine, however, are not the only

[277] organs primarily concerned in anaphylaxis, is shown by certain modifications of the above experiments. In the partially eviscerated animals, and in the supra-diaphragmatic half of the intact animal, in which no reaction takes place on a serum injection, it was above stated that a shock *might* develop on releasing the ligatures. It was not stated, however, that this reaction would *always* take place.

If the ligatures are kept closed but two or three minutes after the serum injection, a shock usually results. If, however, the ligatures are not released till a later period, no shock develops, although a second serum injection now, with the ligatures open, will give a typical reaction.

This shows that there is, either in the blood itself, or in the non-reacting fixed tissues, an efficient mechanism for the destruction, elimination, binding, or for otherwise rendering the injected serum anaphylactically inactive. That this anti-anaphylactic mechanism is probably not wholly situated in the blood itself, is indicated by experiments, in which a sufficient serum dose was mixed with drawn blood, the mixtures defibrinated, and reinjected about ten minutes later. On this reinjection a shock developed.

Which of the fixed body cells cooperate with the blood in producing this anti-anaphylactic effect, I have not as yet attempted to determine; but work is being planned under the hypothesis that the capillary endothelium is the principal tissue concerned in this action.

I believe I can best summarize the view of anaphylaxis necessary from the above work by defining the *acute anaphylactic reaction in dogs as an explosive auto-intoxication of hepatic and intestinal origin, which auto-intoxication is modified, inhibited and overcome, by a more or less efficient anti-anaphylactic mechanism, part, at least, of which is situated in other organs.*

As to the nature of this hypothetical auto-intoxication, whether it consists of split or conjugation products of the injected proteid, of liberated hepatic or intestinal enzymes, of an unusual amount of the normal internal secretion of these organs, or of a special internal secretion peculiar to anaphylaxis, can only be determined, after extensive histological,

chemical and bio-chemical studies. The facts at present at our [277]
command do not warrant the formation of even a working hypothesis on this point.

I believe, however, that the facts above presented have a broader bearing on serum theory than their meaning in anaphylaxis. The anaphylactic material in the body clearly has its meaning, largely in terms of the functional activities of certain fixed body cells with which it cooperates. It is possible that the other substances with which we have been dealing in serology, such as the precipitins, the antitoxins, and the like, are also more complex in their reaction than usually assumed, and involve the cooperation of numerous fixed tissues. Should this be true, it would necessitate a revision of practically all our present serum concepts.

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